Risk factors for the development of Achilles tendinopathy

Evi Wezenbeek

ISBN
978-94-028-1093-6

Design and layout
In Zicht Grafisch Ontwerp, www.promotie-inzicht.nl

Print
Ipskamp Printing, Enschede

Risk factors for the development of Achilles tendinopathy

Evi Wezenbeek

Thesis submitted to fulfill the requirements for the degree of
Doctor in Health Sciences

Ghent University, 2018

Department of Rehabilitation Sciences and Physiotherapy
Faculty of Medicine and Health Sciences

GHENT
UNIVERSITY

Promotor

Prof. Dr. Erik Witvrouw	Ghent University, Ghent, Belgium

Co-promotor

Prof. Dr. Dirk De Clercq	Ghent University, Ghent, Belgium

Process Supervisory Board

Prof. Dr. Martine De Muynck	Ghent University Hospital, Ghent, Belgium
Dr. Nele Mahieu	Ghent University, Ghent, Belgium
Prof. Dr. Luc Vanden Bossche	Ghent University Hospital, Ghent, Belgium

Examination Board

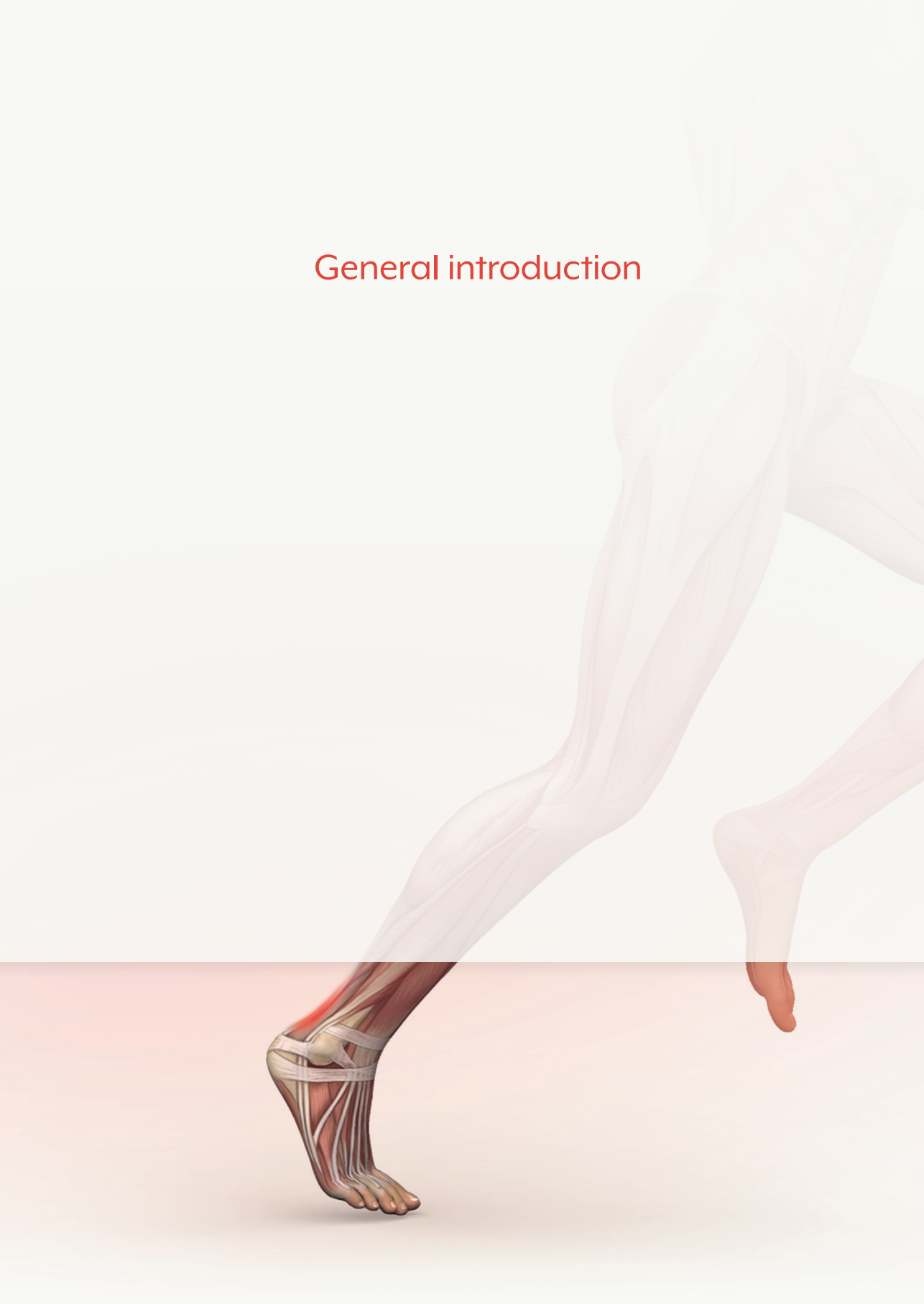
Prof. Dr. Jill Cook	La Trobe University, Melbourne, Australia
Prof. Dr. Hans Zwerver	University Medical Center, Groningen, The Netherlands
Prof. Dr. Dirk Cambier	Ghent University, Ghent, Belgium
Prof. Dr. Ann Cools	Ghent University, Ghent, Belgium
Prof. Dr. Philip Roosen	Ghent University, Ghent, Belgium
Prof. Dr. Adelheid Steyaert	Ghent University Hospital, Ghent, Belgium

Optimism is the faith that leads to achievement;
nothing can be done without hope and confidence.

Helen Keller

Table of contents

General introduction



General introduction

Tendinopathy, the most prevalent tendon disorder, is considered an important overuse injury of the tendon. It is common in sports and its prevalence is ever-increasing.[2, 43, 64] In this dissertation, the focus is on Achilles tendinopathy since the Achilles tendon is one of the most frequently injured tendons in the human body despite its strength.[67] Since prevention is imperative, this dissertation investigates possible risk factors and contributing factors in the development of Achilles tendinopathy. The introduction of this thesis starts with an explanation of healthy tendon structure and function. Then, epidemiology and the clinical impact of Achilles tendinopathy is described, followed by the clinical diagnosis and Imaging of Achilles tendinopathy. Next, the pathophysiologic process of tendinopathy is included, and previously identified risk factors for Achilles tendinopathy are listed. Subsequently the aims of this dissertation are proposed in the introduction.

Achilles tendinopathy

Healthy Achilles tendon

The Achilles tendon is the thickest and strongest tendon in the human body.[47] The Achilles tendon is the combination of tendons of soleus and gastrocnemius muscles, inserting approximately halfway the calcaneus. The gastrocnemius muscle crosses the knee, subtalar and ankle joints, originating from the posterior medial and lateral femoral condyles and inserting onto the calcaneus. The soleus muscle lies anterior to the gastrocnemius muscle and originates from the proximal tibia, fibula and interosseous membrane and crosses the ankle and subtalar joints. Distally, both the gastrocnemius and soleus muscles form an aponeurosis, from each of which a tendon originates. At about the level where the soleus contributes fibers to the Achilles tendon, rotation of the tendon begins and becomes more marked 2–6 cm proximal to the insertion onto the calcaneus. The gastrocnemius fibers rotate to lateral and the soleus fibers are positioned medial to the insertion.[81]

A normal tendon includes three main components: (1) type I collagen fibers, which are predominantly parallel orientated and well-organized, providing high tensile tendon strength[48]; (2) a well-hydrated, extracellular matrix (rich in glycosamino-glycans); and (3) cells.[95] The predominant cell population in healthy tendon is traditionally categorized as fibroblasts, responsible for the synthesis of the extracellular matrix and collagen fibers.[37] In healthy Achilles tendons, no significant renewal of the collagen matrix seems to occur during adult life since Heinemeier et a.[39]measured 14C content within the tendon and concluded that tissue turnover,

Fig. 1 The rotation of the Achilles tendon in a right limb (dorsal view).

indicating tendon adaptability, is mostly seen during adolescence and is limited hereafter. A collagen fibril is the smallest entity of a tendon. The Achilles tendon comprises bundles of collagen fibrils, each wrapped in endotenon, which in turn are enveloped by an epitenon. Further protection and stability is provided to the Achilles tendon by the paratenon, a layer of thin, areolar tissue wrapped around the epitenon.[111] This hierarchical tendon structure is illustrated in Figure 2.

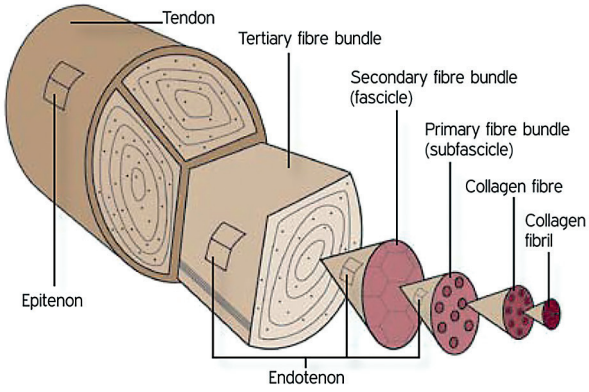


Fig. 2 The hierarchical structure of tendon (www.sportEX.net).

The Achilles tendon is supplied by both the posterior tibial and the peroneal artery.[3] The vessels of the anterior paratenon, deriving from the posterior tibial artery, supply the length of the tendon. Also, the peroneal artery, probably through anastomoses with the posterior tibial artery, makes small contributions and the anterior tibial artery appears not to be involved.[102] Vascular supply of the Achilles tendon has originally thought to be poor, but more recent studies state that Achilles tendon blood flow is adequate for metabolic demands.[1] The neural supply to the Achilles tendon and the surrounding paratenon is provided by nerves from the attaching muscles and by small fasciculi from cutaneous nerves, in particular the sural nerve.[1] The nerve endings in tendons include Ruffini corpuscle and Golgi tendon organ mechanoreceptors as well as free nerve endings that serve a nociceptive function.[90]

The function of a tendon is to attach muscle to bone, thereby transferring force of the contracting muscle to bone and either inducing or controlling movement.[90] The Achilles tendon is part of a musculotendinous unit that spans three joints, producing knee flexion, plantarflexion and subtalar inversion (exerting a medial pull on the calcaneus).[106] It is one of the most frequently and highest loaded tendons in the human body, especially during physical weight bearing activities such as running, walking, jumping et cetera.[44, 55, 57] In vivo experiments have measured peak forces for Achilles tendon ranging from 3kN to 9kN exceeding 12 times the body weight during these sport activities.[54] A healthy tendon can adapt to various forms of physical activity through communication of the cells and cell-driven adaptation of the matrix, this is called mechanotransduction.[51] The Achilles tendon is an energy storage tendon, showing a large capacity to store and release energy. During concentric contractions the function of the tendon is the transmission of force from the muscle fibers to the bone, while during eccentric contractions the tendon stores elastic energy.[103] During the stretch-shortening cycle when an eccentric contraction is immediately followed by a concentric contraction, this stored energy is thought to contribute to the force generation and mechanical efficiency of the movement.[56] In repetitive hopping for instance, most of the elastic energy is stored in the Achilles tendon itself. The contribution of the elastic energy in hopping is about 40% of the total mechanical work.[33, 59] This implies that the tendon plays a very important role during locomotion and sport.[64]

Epidemiology and Clinical impact

Achilles tendinopathy (AT) is one of the most prevalent overuse tendon injuries.[90] Typically, this injury is more prevalent among track athletes, distance runners and individuals who participate in multidirectional sports such as volleyball, basketball, soccer and badminton.[28, 43, 58, 85] Studies show that a prevalence up to 36% has

been reported for AT in adult runners.[40, 62] However, AT is also seen in individuals who are not physically active.[6] The highest incidence for AT is usually reported to occur in middle-age recreational male athletes.[5, 67, 82] It should be mentioned that the prevalence of AT is probably an underestimation since time-loss is often taken into account for diagnosis, while more recent studies found that tendon injuries are not adequately captured using a traditional time-loss definition.[23]

The treatment, that usually consists of relative rest and load management, makes the rehabilitation of tendinopathy a time-consuming process,[86] with a median recovery time of 82 days (ranging from 21-479 days).[79] Furthermore, the prognosis is often poor, with a high incidence of recurrence.[18, 31, 49] Reinjury rates of AT in soccer players range from 27% to 44%.[34, 38, 97] The high recurrence rate for AT and the little evidence on how to prevent these recurrences,[34] may indicate that other strategies should be considered in the rehabilitation approach.

AT, associated with persistent pain, significantly affects the patients physical and psychosocial well-being.[73] Furthermore, the consequences of this overuse injury constitute a major influence on an athlete's sports career and quality of life.[10, 60] Since AT causes considerable morbidity and functional impairment among athletic and general populations,[42] improving knowledge on development and prevention of AT will contribute to a healthy ageing population.

Clinical diagnosis and Imaging

The term tendinopathy is the description of tendon conditions encompassing pain (mostly during activity), possible palpable thickening, and impaired performance.[65] Clinical examination is the best diagnostic tool, and pain is the cardinal symptom of AT.[66] Also, a sensation of morning stiffness and tenderness to palpation is frequently reported by these patients.[19, 97] AT most often occurs at the midportion of the Achilles tendon, located 2-6 cm proximal to the calcaneal insertion, followed by insertional Achilles disorders (at the calcaneus-Achilles tendon junction).[27, 43, 63]

Achilles tendinopathy is the most common cause of posterior heel pain; other tendinopathies (plantaris, flexor hallucis longus, tibialis posterior and the peroneal tendons) result in pain localized at the affected tendon. Pain location of plantaris tendon pain is medial, where the plantaris sits adjacent to the Achilles. Pain is typically 6-8 cm above the Achilles insertion, where the plantaris merges with the Achilles tendon and its tendon sheath. Midportion Achilles tendinopathy will be slightly lower in the body of the tendon, whilst insertional Achilles tendinopathy will be much lower at the insertion point of the Achilles on the calcaneus. Posterior heel pain can also be attributed to Haglund deformity (a prominence of the

calcaneus that may lead to retrocalcaneal bursa inflammation) or Sever disease (calcaneal apophysitis common in children and adolescents). Also, Inflammation at the enthesis, i.e. enthesitis, is a characteristic feature of spondyloarthropathy (a general inflammatory disease).[91, 105, 110]

Ultrasound (US) imaging is often used to confirm the clinical diagnosis of AT,[72] since this is more popular than MRI among musculoskeletal practitioners by offering advantages as minimal invasive, more affordable and being quick and feasible to use.[22, 60] A conventional ultrasound image can show anterior-posterior diameter, presence of a hypoechoic area, calcifications and peritendinous abnormalities, whereas Doppler ultrasound can image vascular ingrowth in the tendon.[72] It should be mentioned that one of the most frequently cited criticisms in relation to US imaging is its reliability since it is perceived to have a higher risk of error or variance when evaluating tendon modalities, except for measuring tendon thickness.[35, 72] Also, Ultrasound elastography (EUS) is a method to assess the mechanical properties of tissue, by applying stress and detecting tissue displacement using ultrasound. There are several EUS techniques used in clinical practice; strain EUS is the most common technique and is stated to be is an accurate clinical tool in the evaluation of Achilles tendinopathy, comparable to US.[25, 83]

Because of a growing interest to quantify tendon integrity, a new imaging technique was developed to visualize tendon structure: Ultrasound Tissue Characterisation (UTC).[108] UTC uses conventional ultrasonography to construct a 3D image of the tendon and dedicated algorithms quantify the stability of echopatterns, whereby four echo-types can be discriminated which reflect the structural integrity of the tendon: (echo-type I) intact and aligned tendon bundles; (echo-type II) discontinuous or waving tendon bundles; (echo-type III) more fibrillar and disorganized tissue structure; and (echo-type IV) amorphous matrix largely composed of fluid,[108] as visualized in Figure 3.

Pathophysiology and pain

The process of tendinopathy involves the collagen matrix and the tenocytes. Normally, collagen fibers in tendons are tightly bundled in a parallel fashion but tendinopathic samples show unequal and irregular crimping, loosening and increased waviness of collagen fibers, with an increase in type III reparative collagen.[50] Next to this disrupted collagen, pathological tendons have proliferation of cells with abnormal rounded nuclei, increased amount of ground substance/ matrix proteins and possibly abnormal ingrowths of vessels and nerves, called neoneurovascularization,[96] as illustrated in Figure 4. Neovascularization is a US-detectable intratendinous vascularization and is frequently associated with tendon disorders

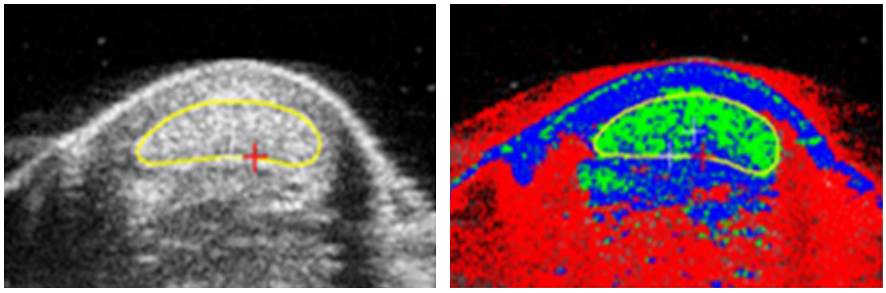


Fig. 3 UTC software. Visualization of a right AT in the transversal view. Echo- type I is colored green, echo-type II is colored blue, echo-type III is colored red, and echo-type IV is colored black.

because of its occurrence in 47% to 88% of patients with Achilles tendinopathy.[93, 104] Doppler studies consistently identify neovessels on the aspect of the tendon immediately adjacent to the neighboring fat pad, namely in the Achilles tendon where neovessels infiltrate the ventral aspect of the tendon.[109] However, a mere association of neovascularization with disorders is questionable,[13, 104] since neovascularization has also been found in up to 35% of asymptomatic Achilles tendons.[14, 40] Furthermore, previous research stated that the detection of neo-

Fig. 4 Structural changes in Achilles tendinopathy (by Scott et al.[96]). The left side of the hypothetical tendon is healthy. The right side represents typical changes in a tendinopathic tendon.

vascularization has no additional value for the diagnosis, no firmly confirmed prognostic value, and no proven relation with symptoms and therefore suggested that the role of neovascularization should be re-thought.[104] More recently, the role of neovascularization shifted from an indicator for pathology to critical for tissue repair and wound healing.[101]

Achilles tendinopathy is associated with impairments such as decreases in motor control, strength, endurance, and plyometric ability.[7, 69, 97] The mechanical properties of the tendon also change with tendinopathy.[8] The tendinopathic tendon has been found to have lower tendon stiffness,[71] which might also affect the force-generating capability of the tendon.[97] This pathological tendon structure is assumed to be less able to tolerate load and more vulnerable to further injury.[113]

Several models have been proposed to explain the pathophysiology of Achilles tendinopathy.[1] A few examples of these models are the 'iceberg' model of Abate et al.,[1] that states that micro-ruptures and neurogenic inflammation exist before presentation of symptoms of tendinopathy, and the 'failed healing' theory of Fu et al.,[32] that proposes a 3-stage model in which multiple factors influence the tendon in three stages: injury, failed healing and eventually clinical presentation of symptoms. The most accepted theoretical patho-etiological model however is the "continuum model".[21] The continuum model of tendon pathology suggested by Cook and Purdam[20] consists of three stages: reactive tendinopathy, tendon disrepair and degenerative tendinopathy, as illustrated in Figure 5. Load magnitude and recovery time, as well as numerous other factors (type and consistency of load stimulus), may influence tendon response and the progression along the continuum.[68] The first stage of the pathology results from acute overload of the Achilles tendon and can be characterized as a non-traditional inflammatory proliferative response in the cell and matrix. The second stage is described as attempted tendon healing, through increased production of collagen and proteoglycans. Degenerative tendinopathy is the third stage and characterized by potentially irreversible changes in cell and matrix condition such as tenocyte apoptosis and matrix disorder.[20] Since there is little capacity for reversibility of pathological changes at this stage,[20] early detection of pathology followed by adequate treatment would be beneficial to inhibit forward progression in the continuum model and prevent recurrences. It is stated that the continuum model uses a structure based classification of tendinopathy.[21] Furthermore, UTC is demonstrated to be able to detect subtle changes in tendon structure.[108] Therefore, it is possible that UTC could be useful in the early detection of alterations in tendon structure, leading to a quick and adequate management, to inhibit forward progression in the continuum or even to prevent symptoms.

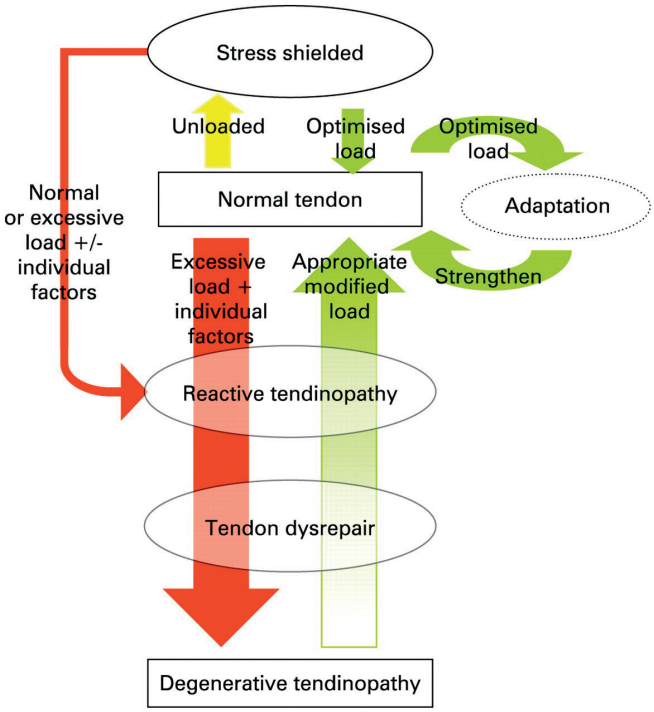


Fig. 5 The continuum model of tendon pathology (by Cook and Purdam[20]).

Achilles tendon pain remains an enigma, although different hypotheses about the origin of pain exist.[92] Originally, the pain of Achilles tendinopathy was attributed to an inflammatory process. While inflammatory cells have been observed, particularly in the early stages of Achilles tendinopathy, the response does not seem to be a traditional inflammatory response.[95] Both local tendon based nociceptive contributors and extensive mechanisms within the periphery and the central nervous system are described in previous research investigating mechanisms for tendon pain.[92] Tendon pain is partly related to function, with tendinopathy decreasing muscle strength and motor control, which in turns reduces function.[98] However, alterations in tendon function also occur in the presence of structural pathology, independent of pain. For instance, it has been shown that athletes with an asymptomatic tendinopathy displayed a different landing strategy compared with their counterparts with normal tendons.[26, 70] This highlights the complex interplay among structure, pain and function.[20]

Despite the high prevalence and the often deleterious consequences of Achilles tendinopathy, many aspects of its aetiology remain unknown. Overuse is generally considered to induce the condition since relative overuse of the tendon may cause an imbalance between anabolic and catabolic processes in the extracellular matrix of the tendon.[52] In this process, both intrinsic and extrinsic factors play an important role.[67, 82] Thus, although load is a major patho-aetiological component, it is modulated by an interaction between intrinsic and extrinsic factors.[20]

Risk factors and Early signs of pathology

Any causal factor increasing the chance of an injury occurring is considered a risk factor.[10] Risk factors are often divided into two main categories: extrinsic and intrinsic factors.[76] Extrinsic risk factors are considered to act on the predisposed athlete from outside and are classified as enabling factors that facilitate the manifestation of injury. Intrinsic risk factors are related to the individual biological and psychological characteristics and determine the load tolerance. Hence, they might make the individual predisposed to injury.[10] These risk factors will determine whether a specific event or force will lead to injury occurrence.[11] To date, and to the best of our knowledge, no distinction for modifiable and non-modifiable risk factors for Achilles tendinopathy has been made. Therefore, the described risk factors below are categorized in extrinsic/modifiable, extrinsic/non-modifiable, intrinsic/modifiable or intrinsic/non-modifiable.

Intrinsic/ non-modifiable	Intrinsic/ modifiable	Extrinsic/ non-modifiable	Extrinsic/ modifiable
Male sex	Tendon vascularity	Environmental factors such as temperature	Steroids & antibiotic exposure
Advancing age	Altered gait kinematics & kinetics, particularly foot pronation		Physical activity-related factors: alteration to activity level
Genetics	Flexibility		Footwear
Systemic diseases and associated factors	Neuromuscular alterations		Training surfaces

In recent years there has been an increase in studies examining different variables as potential risk factors for the development of AT.[67] The majority of these are cross sectional, termed association studies rather than longitudinal studies, which are

often prospective in nature. The two types of studies have different uses. Association studies develop information about relationships between diseases and variables whilst prospective studies allow some measure of the cause and effect relationship to be established.[82] The current literature suggests that risk factors in the development of AT may include *male sex* [46, 61] and *advancing age*.[61, 64, 112] These factors have not been prospectively examined[82] but are suggested since the highest incidence for AT is usually reported to occur in middle-age recreational male athletes.[4, 5, 67, 82] These suggested risk factors have been previously explained by the association of age with increased prevalence of degenerative changes, such as decreased cellularity, increased glycosaminoglycan content, lack of fiber organization,[67] and since estrogen is suggested to affect tendon structure positively.[17]

Another frequently suggested risk factor is *tendon vascularity*, since it is suggested that in a hypovascular tendon the extent of necessary matrix remodeling is restricted, which may lead to weakening of the tendon structure, causing tendinopathy.[87, 89] Although 23 articles indicate a restricted blood supply, and its correlation to the pathogenic role of hypoxia, as an important risk factor,[67] it remains unclear whether this predicts future symptoms. These studies reported the need for longitudinal studies to investigate the physiological or pathological effect of vascularization, especially after physical activity, and its predictive value in the development of AT.[14, 80]

Next, *altered gait kinematics and kinetics*, particularly foot pronation, are frequently hypothesized to be a risk factor for AT.[78] It has been suggested that excessive foot pronation creates an accentuated rotation of the Achilles tendon, which is defined as the "whipping phenomenon", as the foot rotates rapidly from an inverted position at heel strike to an excessively everted position in midstance, and that this wringing out of the tendon results in vascular blanching of the midportion of the tendon, causing AT.[15, 88, 99] This whipping hypothesis is supported by more recent studies that demonstrated an association between pronation and AT,[24, 74, 94] since they found that subjects with AT showed greater pronation during running than controls. However, prospective studies are needed and further evidence should be provided to support the theory that this accentuated rotation causes a wringing of the Achilles tendon that is associated with AT.[78]

Furthermore, *recent injuries or previous lower limb tendinopathy tendinopathy*[2, 82] are indicated by experts in a Delphi study as the prime risk factors for the development of AT. Also, *flexibility*, including a decreased flexibility of the gastrocnemius/soleus complex and an altered ankle dorsiflexion range of motion, have been proposed as

intrinsic risk factors for AT. [70, 89] In addition, *altered neuromuscular control* of the triceps surae has been indicated to play a role in AT. [39, 70] Reduced isokinetic plantarflexion strength has been reported in subjects with AT, as has decreased EMG amplitude of the triceps surae just after heel strike and push-off.[12, 113] Other suggested intrinsic risk factors are *systemic diseases and associated factors* like diabetes mellitus,[1, 13] rheumatologic disease,[44] dislipidemia,[96] hypertension,[42] obesity,[33] genetics,[91] hormone replacement therapy and menopause.[18]

As extrinsic risk factors, *steroid and antibiotic exposure (more specific quinolones)* [100, 112] and *physical activity-related factors*; including "training errors" and alterations to activity levels, footwear and environmental factors such as temperature and training surfaces [9, 53, 63, 77] are described to be involved.

Because of conflicting results of longitudinal studies regarding *Achilles tendon structure* as risk factor for AT, it is possible that structural changes in asymptomatic populations may represent markers of early presymptomatic pathology.[73] Given the substantial impact of AT, this identification of 'early signs of pathology' should be a priority, and further research is warranted. As stated, several studies have investigated whether tendon structure in asymptomatic individuals, visualized by US, is predictive for future symptoms, and showed conflicting results.[13, 16, 30, 36, 41, 45, 84] A relationship between the presence of Achilles tendon abnormalities, more specifically hypoechoic regions, and the development of future symptoms was reported by both Comin et al.[16] and Fredberg et al.,[30] whereas Giombini et al.[36] and Ooi et al.[84] could not support this finding. Hirschmuller et al.[41] found that the presence of neovascularization was associated with increased risk of developing symptoms, whereas Boesen et al.,[13] Comin et al.[16] and Ooi et al.[84] did not find this. Also, Jhinghan et al.[45] and Ooi et al.[84] found that tendon thickness in asymptomatic individuals is predictive for future symptoms, but this finding is in contrast to Comin et al.,[16] Giombini et al.[36] and Hirschmuller et al.[41] who found no relationship between tendon thickness and the development of symptomatic AT.

Unfortunately many of these reported risk factors are not based on primary epidemiological data and often represent author opinion. Two systematic reviews on risk factors for AT exist with one addressing biomechanical alterations[78] and the other focusing solely on runners.[63] Although several associating factors have been proposed (e.g. increased BMI, adverse lipid profile, certain genetic markers, altered plantar loading, altered neuromuscular control and increased rearfoot eversion), neither of these reviews could identify strong risk factors since the evidence is weak for most variables and there is a lack of high quality studies.[63, 78] Furthermore, only prospective studies can be used to make conclusions about causality, but

unfortunately there are only few prospective studies investigating risk factors for the development of AT.[63, 78, 82] Next to their crucial role in (secondary) prevention,[75] these risk factors may alter the progression forward or back in the continuum and most are likely to have an important role in the response to treatment in tendinopathy.[20] Therefore, further research is needed to address this gap.

Background and aims

Additional research on AT is imperative because of the lack of knowledge of its complex aetiology and pathophysiology, and the high prevalence of AT with clinical impact on activities of daily living and sports remains. Understanding the cause of injury is a necessity to advance knowledge concerning prediction and prevention.[29, 107] As a consequence, this dissertation aimed to better understand demographic, structural, biomechanical and vascular parameters of AT and to prospectively investigate suggested intrinsic risk factors for the development of AT. Since AT most often occurs at the midportion of the Achilles tendon,[27, 63] this project focused on midportion AT.

Specific aim of this thesis are:

1. To better understand demographic, structural, biomechanical and vascular parameters of AT
2. To prospectively investigate suggested risk factors for the development of AT

AIM 1: To better understand demographic, structural, biomechanical and vascular parameters of AT

In chapters I and II we investigated tendon structure and the whipping phenomenon in a healthy population since there is a need for better knowledge of the normal Achilles tendon structure and the whipping phenomenon before linking this to AT. Regarding tendon structure, the aforementioned UTC technique was used. Because of increasing publications using UTC, there is a need for normative data in order to correctly analyze results of UTC studies. Moreover, these normative data are imperative before being able to detect aberrant tendon structure. Furthermore, it remains unknown whether sex or dominance affect the echo-type distribution in the Achilles tendon. This normal tendon characterization was investigated in chapter I. In chapter II, the whipping phenomenon was studied since this mechanism is frequently suggested to contribute to the etiology of AT.[15] However, despite (cross-sectional) evidence that links pronation to the onset of AT,[15, 24, 74, 94] this underlying injury mechanism was surprisingly never investigated before. Next, since the highest incidence for AT is reported to occur in middle-aged male athletes, chapter IV examined the role of age and sex on an established risk factor in the development of AT.

AIM 2: To prospectively investigate suggested risk factors for the development of AT

The pathogenesis pathways of AT are heterogeneous. Both structural and vascular factors have been suggested as risk factors for the development of AT. However, although tendon vascularity is often suggested as risk factor, longitudinal studies are necessary to investigate the predictive value in development of tendinopathy.[14] Because of the cross-sectional evidence that links pronation -whether or not associated to blood flow- to AT, the role of foot posture in the development of AT was also investigated in this project. Furthermore, there is inconclusive evidence on the role of tendon structure, visualized by US, in the detection of early signs of pathology and the prediction of future symptoms. Since UTC is suited to quantify subtle tendon structure changes,[108] the hypothesis is that UTC could be useful in the early detection of AT. Despite increasing publications on the use of UTC, no prospective studies have been published. Therefore, in chapter III, we prospectively investigated the role of the vascular response to activity, foot posture, Achilles tendon thickness and UTC tendon structure as possible risk factors in the development of AT. Further elucidation of risk factors and early signs of detection will aid in the understanding of tendon pathology and patient risk, thereby improving prevention for AT.

References

1. Abate M, Silbernagel KG, Siljeholm C, et al. Pathogenesis of tendinopathies: inflammation or degeneration? *Arthritis Res Ther.* 2009;11(3):235.
2. Ackermann PW, Renstrom P. Tendinopathy in sport. *Sports Health.* 2012;4(3):193-201.
3. Ahmed IM, Lagopoulos M, McConnell P, Soames RW, Sefton GK. Blood supply of the Achilles tendon. *J Orthop Res.* 1998;16(5):591-596.
4. Albers IS, Zwerver J, Diercks RL, Dekker JH, Van den Akker-Scheek I. Incidence and prevalence of lower extremity tendinopathy in a Dutch general practice population: a cross sectional study. *BMC Musculoskelet Disord.* 2016;17:16.
5. Alfredson H, Bjur D, Thorsen K, Lorentzon R, Sandstrom P. High intratendinous lactate levels in painful chronic Achilles tendinosis. An investigation using microdialysis technique. *J Orthop Res.* 2002;20(5):934-938.
6. Alfredson H, Lorentzon R. Chronic Achilles tendinosis: recommendations for treatment and prevention. *Sports Med.* 2000;29(2):135-146.
7. Alfredson H, Pietila T, Lorentzon R. Chronic Achilles tendinitis and calf muscle strength. *Am J Sports Med.* 1996;24(6):829-833.
8. Arya S, Kulig K. Tendinopathy alters mechanical and material properties of the Achilles tendon. *J Appl Physiol (1985).* 2010;108(3):670-675.
9. Azevedo LB, Lambert MI, Vaughan CL, O'Connor CM, Schwellnus MP. Biomechanical variables associated with Achilles tendinopathy in runners. *Br J Sports Med.* 2009;43(4):288-292.
10. Bahr R. No injuries, but plenty of pain? On the methodology for recording overuse symptoms in sports. *Br J Sports Med.* 2009;43(13):966-972.
11. Bahr R, Krosshaug T. Understanding injury mechanisms: a key component of preventing injuries in sport. *Br J Sports Med.* 2005;39(6):324-329.
12. Baur H, Muller S, Hirschmuller A, Cassel M, Weber J, Mayer F. Comparison in lower leg neuromuscular activity between runners with unilateral mid-portion Achilles tendinopathy and healthy individuals. *J Electromyogr Kinesiol.* 2011;21(3):499-505.
13. Boesen AP, Boesen MI, Torp-Pedersen S, et al. Associations between abnormal ultrasound color Doppler measures and tendon pain symptoms in badminton players during a season: a prospective cohort study. *Am J Sports Med.* 2012;40(3):548-555.
14. Cassel M, Baur H, Hirschmuller A, Carlsohn A, Frohlich K, Mayer F. Prevalence of Achilles and patellar tendinopathy and their association to intratendinous changes in adolescent athletes. *Scand J Med Sci Sports.* 2015;25(3):e310-318.
15. Clement DB, Taunton JE, Smart GW. Achilles tendinitis and peritendinitis: etiology and treatment. *Am J Sports Med.* 1984;12(3):179-184.
16. Comin J, Cook JL, Malliaras P, et al. The prevalence and clinical significance of sonographic tendon abnormalities in asymptomatic ballet dancers: a 24-month longitudinal study. *Br J Sports Med.* 2013;47(2):89-92.
17. Cook JL, Bass SL, Black JE. Hormone therapy is associated with smaller Achilles tendon diameter in active post-menopausal women. *Scand J Med Sci Sports.* 2007;17(2):128-132.
18. Cook JL, Khan KM, Harcourt PR, Grant M, Young DA, Bonar SF. A cross sectional study of 100 athletes with jumper's knee managed conservatively and surgically. The Victorian Institute of Sport Tendon Study Group. *Br J Sports Med.* 1997;31(4):332-336.
19. Cook JL, Khan KM, Purdam C. Achilles tendinopathy. *Man Ther.* 2002;7(3):121-130.
20. Cook JL, Purdam CR. Is tendon pathology a continuum? A pathology model to explain the clinical presentation of load-induced tendinopathy. *Br J Sports Med.* 2009;43(6):409-416.
21. Cook JL, Rio E, Purdam CR, Docking SI. Revisiting the continuum model of tendon pathology: what is its merit in clinical practice and research? *Br J Sports Med.* 2016;50(19):1187-1191.
22. Docking SI, Ooi CC, Connell D. Tendinopathy: Is Imaging Telling Us the Entire Story? *J Orthop Sports Phys Ther.* 2015;45(11):842-852.

23. Docking SI, Rio E, Cook J, Orchard JW, Fortington LV. The prevalence of Achilles and patellar tendon injuries in Australian football players beyond a time-loss definition. *Scand J Med Sci Sports.* 2018.
24. Donoghue OA, Harrison AJ, Laxton P, Jones RK. Orthotic control of rear foot and lower limb motion during running in participants with chronic Achilles tendon injury. *Sports Biomech.* 2008;7(2):194-205.
25. Drakonaki EE, Allen GM, Wilson DJ. Ultrasound elastography for musculoskeletal applications. *Br J Radiol.* 2012;85(1019):1435-1445.
26. Edwards S, Steele JR, McGhee DE, Beattie S, Purdam C, Cook JL. Landing strategies of athletes with an asymptomatic patellar tendon abnormality. *Med Sci Sports Exerc.* 2010;42(11):2072-2080.
27. Egger AC, Berkowitz MJ. Achilles tendon injuries. *Curr Rev Musculoskelet Med.* 2017;10(1):72-80.
28. Fahlstrom M, Lorentzon R, Alfredson H. Painful conditions in the Achilles tendon region: a common problem in middle-aged competitive badminton players. *Knee Surg Sports Traumatol Arthrosc.* 2002;10(1):57-60.
29. Finch C. A new framework for research leading to sports injury prevention. *J Sci Med Sport.* 2006;9(1-2):3-9; discussion 10.
30. Fredberg U, Bolvig L. Significance of ultrasonographically detected asymptomatic tendinosis in the patellar and achilles tendons of elite soccer players: a longitudinal study. *Am J Sports Med.* 2002;30(4):488-491.
31. Fredberg U, Bolvig L, Andersen NT, Stengaard-Pedersen K. Ultrasonography in evaluation of Achilles and patella tendon thickness. *Ultraschall Med.* 2008;29(1):60-65.
32. Fu SC, Rolf C, Cheuk YC, Lui PP, Chan KM. Deciphering the pathogenesis of tendinopathy: a three-stages process. *Sports Med Arthrosc Rehabil Ther Technol.* 2010;2:30.
33. Fukashiro S, Komi PV, Jarvinen M, Miyashita M. In vivo Achilles tendon loading during jumping in humans. *Eur J Appl Physiol Occup Physiol.* 1995;71(5):453-458.
34. Gajhede-Knudsen M, Ekstrand J, Magnusson H, Maffulli N. Recurrence of Achilles tendon injuries in elite male football players is more common after early return to play: an 11-year follow-up of the UEFA Champions League injury study. *Br J Sports Med.* 2013;47(12):763-768.
35. Gellhorn AC, Carlson MJ. Inter-rater, intra-rater, and inter-machine reliability of quantitative ultrasound measurements of the patellar tendon. *Ultrasound Med Biol.* 2013;39(5):791-796.
36. Giombini A, Dragoni S, Di Cesare A, Di Cesare M, Del Buono A, Maffulli N. Asymptomatic Achilles, patellar, and quadriceps tendinopathy: a longitudinal clinical and ultrasonographic study in elite fencers. *Scand J Med Sci Sports.* 2013;23(3):311-316.
37. Gross MT. Chronic tendinitis: pathomechanics of injury, factors affecting the healing response, and treatment. *J Orthop Sports Phys Ther.* 1992;16(6):248-261.
38. Hagglund M, Walden M, Ekstrand J. UEFA injury study--an injury audit of European Championships 2006 to 2008. *Br J Sports Med.* 2009;43(7):483-489.
39. Heinemeier KM, Schjerling P, Heinemeier J, Magnusson SP, Kjaer M. Lack of tissue renewal in human adult Achilles tendon is revealed by nuclear bomb (14)C. *FASEB J.* 2013;27(5):2074-2079.
40. Hirschmuller A, Frey V, Deibert P, et al. [Achilles tendon power Doppler sonography in 953 long distance runners - a cross sectional study]. *Ultraschall Med.* 2010;31(4):387-393.
41. Hirschmuller A, Frey V, Konstantinidis L, et al. Prognostic value of Achilles tendon Doppler sonography in asymptomatic runners. *Med Sci Sports Exerc.* 2012;44(2):199-205.
42. Intziegianni K, Cassel M, Rauf S, et al. Influence of Age and Pathology on Achilles Tendon Properties During a Single-leg Jump. *Int J Sports Med.* 2016;37(12):973-978.
43. Jarvinen TA, Kannus P, Maffulli N, Khan KM. Achilles tendon disorders: etiology and epidemiology. *Foot Ankle Clin.* 2005;10(2):255-266.
44. Jarvinen TA, Kannus P, Paavola M, Jarvinen TL, Jozsa L, Jarvinen M. Achilles tendon injuries. *Curr Opin Rheumatol.* 2001;13(2):150-155.
45. Jhingan S, Perry M, O'Driscoll G, et al. Thicker Achilles tendons are a risk factor to develop Achilles tendinopathy in elite professional soccer players. *Muscles Ligaments Tendons J.* 2011;1(2):51-56.
46. Jones BH, Bovee MW, Harris JM, 3rd, Cowan DN. Intrinsic risk factors for exercise-related injuries among male and female army trainees. *Am J Sports Med.* 1993;21(5):705-710.

47. Jozsa L, Kannus P. Histopathological findings in spontaneous tendon ruptures. *Scand J Med Sci Sports*. 1997;7(2):113-118.
48. Kannus P. Etiology and pathophysiology of chronic tendon disorders in sports. *Scand J Med Sci Sports*. 1997;7(2):78-85.
49. Kettunen JA, Kvist M, Alanen E, Kujala UM. Long-term prognosis for jumper's knee in male athletes. A prospective follow-up study. *Am J Sports Med*. 2002;30(5):689-692.
50. Khan KM, Cook JL, Bonar F, Harcourt P, Astrom M. Histopathology of common tendinopathies. Update and implications for clinical management. *Sports Med*. 1999;27(6):393-408.
51. Khan KM, Scott A. Mechanotherapy: how physical therapists' prescription of exercise promotes tissue repair. *Br J Sports Med*. 2009;43(4):247-252.
52. Kjaer M, Langberg H, Miller BF, et al. Metabolic activity and collagen turnover in human tendon in response to physical activity. *J Musculoskelet Neuronal Interact*. 2005;5(1):41-52.
53. Knobloch K, Yoon U, Vogt PM. Acute and overuse injuries correlated to hours of training in master running athletes. *Foot Ankle Int*. 2008;29(7):671-676.
54. Komi PV. Relevance of in vivo force measurements to human biomechanics. *J Biomech*. 1990;23 Suppl 1:23-34.
55. Kubo K, Ikebukuro T, Tsunoda N, Kanehisa H. Changes in oxygen consumption of human muscle and tendon following repeat muscle contractions. *Eur J Appl Physiol*. 2008;104(5):859-866.
56. Kubo K, Kanehisa H, Fukunaga T. Comparison of elasticity of human tendon and aponeurosis in knee extensors and ankle plantar flexors in vivo. *J Appl Biomech*. 2005;21(2):129-142.
57. Kubo K, Tabata T, Ikebukuro T, Igarashi K, Yata H, Tsunoda N. Effects of mechanical properties of muscle and tendon on performance in long distance runners. *Eur J Appl Physiol*. 2010;110(3):507-514.
58. Kvist M. Achilles tendon injuries in athletes. *Sports Med*. 1994;18(3):173-201.
59. Lamontagne M, Kennedy MJ. The biomechanics of vertical hopping: a review. *Res Sports Med*. 2013;21(4):380-394.
60. Lian OB, Engebretsen L, Bahr R. Prevalence of jumper's knee among elite athletes from different sports: a cross-sectional study. *Am J Sports Med*. 2005;33(4):561-567.
61. Longo UG, Ronga M, Maffulli N. Achilles tendinopathy. *Sports Med Arthrosc Rev*. 2009;17(2):112-126.
62. Lopes AD, Hespanhol Junior LC, Yeung SS, Costa LO. What are the main running-related musculoskeletal injuries? A Systematic Review. *Sports Med*. 2012;42(10):891-905.
63. Lorimer AV, Hume PA. Achilles tendon injury risk factors associated with running. *Sports Med*. 2014;44(10):1459-1472.
64. Maffulli N, Kenward MG, Testa V, Capasso G, Regine R, King JB. Clinical diagnosis of Achilles tendinopathy with tendinosis. *Clin J Sport Med*. 2003;13(1):11-15.
65. Maffulli N, Regine R, Angelillo M, Capasso G, Filice S. Ultrasound diagnosis of Achilles tendon pathology in runners. *Br J Sports Med*. 1987;21(4):158-162.
66. Maffulli N, Wong J, Almekinders LC. Types and epidemiology of tendinopathy. *Clin Sports Med*. 2003;22(4):675-692.
67. Magnan B, Bondi M, Pierantoni S, Samaila E. The pathogenesis of Achilles tendinopathy: a systematic review. *Foot Ankle Surg*. 2014;20(3):154-159.
68. Magnusson SP, Langberg H, Kjaer M. The pathogenesis of tendinopathy: balancing the response to loading. *Nat Rev Rheumatol*. 2010;6(5):262-268.
69. Magnusson SP, Narici MV, Maganaris CN, Kjaer M. Human tendon behaviour and adaptation, in vivo. *J Physiol*. 2008;586(1):71-81.
70. Mann KJ, Edwards S, Drinkwater EJ, Bird SP. A lower limb assessment tool for athletes at risk of developing patellar tendinopathy. *Med Sci Sports Exerc*. 2013;45(3):527-533.
71. Maquirriain J. Leg stiffness changes in athletes with Achilles tendinopathy. *Int J Sports Med*. 2012;33(7):567-571.
72. Mc Auliffe S, Mc Creesh K, Purtill H, O'Sullivan K. A systematic review of the reliability of diagnostic ultrasound imaging in measuring tendon size: Is the error clinically acceptable? *Phys Ther Sport*. 2017;26:52-63.

73. Mc Auliffe S, Synott A, Casey H, Mc Creesh K, Purtill H, O'Sullivan K. Beyond the tendon: Experiences and perceptions of people with persistent Achilles tendinopathy. *Musculoskelet Sci Pract.* 2017;29:108-114.
74. McCrory JL, Martin DF, Lowery RB, et al. Etiologic factors associated with Achilles tendinitis in runners. *Med Sci Sports Exerc.* 1999;31(10):1374-1381.
75. Meeuwisse WH. Predictability of sports injuries. What is the epidemiological evidence? *Sports Med.* 1991;12(1):8-15.
76. Meeuwisse WH. Assessing Causation in Sport Injury: A Multifactorial Model. *Clinical Journal of Sport Medicine.* 1994;4(3):166-170.
77. Milgrom C, Finestone A, Zin D, Mandel D, Novack V. Cold weather training: a risk factor for Achilles paratendinitis among recruits. *Foot Ankle Int.* 2003;24(5):398-401.
78. Munteanu SE, Barton CJ. Lower limb biomechanics during running in individuals with achilles tendinopathy: a systematic review. *J Foot Ankle Res.* 2011;4:15.
79. Nielsen RO, Ronnow L, Rasmussen S, Lind M. A prospective study on time to recovery in 254 injured novice runners. *PLoS One.* 2014;9(6):e99877.
80. Notarnicola A, Maccagnano G, Di Leo M, Tafuri S, Moretti B. Overload and neovascularization of Achilles tendons in young artistic and rhythmic gymnasts compared with controls: an observational study. *Musculoskelet Surg.* 2014;98(2):115-120.
81. O'Brien M. The anatomy of the Achilles tendon. *Foot Ankle Clin.* 2005;10(2):225-238.
82. O'Neill S, Watson PJ, Barry S. A Delphi Study of Risk Factors for Achilles Tendinopathy- Opinions of World Tendon Experts. *Int J Sports Phys Ther.* 2016;11(5):684-697.
83. Ooi CC, Schneider ME, Malliaras P, Chadwick M, Connell DA. Diagnostic performance of axial-strain sonoelastography in confirming clinically diagnosed Achilles tendinopathy: comparison with B-mode ultrasound and color Doppler imaging. *Ultrasound Med Biol.* 2015;41(1):15-25.
84. Ooi CC, Schneider ME, Malliaras P, et al. Sonoelastography of the Achilles Tendon: Prevalence and Prognostic Value Among Asymptomatic Elite Australian Rules Football Players. *Clin J Sport Med.* 2016;26(4):299-306.
85. Paavola M, Kannus P, Jarvinen TA, Khan K, Jozsa L, Jarvinen M. Achilles tendinopathy. *J Bone Joint Surg Am.* 2002;84-A(11):2062-2076.
86. Peters JA, Zwerver J, Diercks RL, Elferink-Gemser MT, van den Akker-Scheek I. Preventive interventions for tendinopathy: A systematic review. *J Sci Med Sport.* 2016;19(3):205-211.
87. Pufe T, Petersen WJ, Mentlein R, Tillmann BN. The role of vasculature and angiogenesis for the pathogenesis of degenerative tendons disease. *Scand J Med Sci Sports.* 2005;15(4):211-222.
88. R M. Achilles tendonitis. *Foot Ankle Clin.* 1997(2):439-456.
89. Rahim M, El Khoury LY, Raleigh SM, et al. Human Genetic Variation, Sport and Exercise Medicine, and Achilles Tendinopathy: Role for Angiogenesis-Associated Genes. *OMICS.* 2016;20(9):520-527.
90. Reinking M. Tendinopathy in athletes. *Phys Ther Sport.* 2012;13(1):3-10.
91. Rio E, Mayes S, Cook J. Heel pain: a practical approach. *Aust Fam Physician.* 2015;44(3):96-101.
92. Rio E, Moseley L, Purdam C, et al. The pain of tendinopathy: physiological or pathophysiological? *Sports Med.* 2014;44(1):9-23.
93. Risch L, Wochatz M, Messerschmidt J, Engel T, Mayer F, Cassel M. Reliability of Evaluating Achilles Tendon Vascularization Assessed With Doppler Ultrasound Advanced Dynamic Flow. *J Ultrasound Med.* 2017.
94. Ryan M, Grau S, Krauss I, Maiwald C, Taunton J, Horstmann T. Kinematic analysis of runners with achilles mid-portion tendinopathy. *Foot Ankle Int.* 2009;30(12):1190-1195.
95. Scott A, Backman LJ, Speed C. Tendinopathy: Update on Pathophysiology. *J Orthop Sports Phys Ther.* 2015;45(11):833-841.
96. Scott A, Huisman E, Khan K. Conservative treatment of chronic Achilles tendinopathy. *CMAJ.* 2011; 183(10):1159-1165.
97. Silbernagel KG, Crossley KM. A Proposed Return-to-Sport Program for Patients With Midportion Achilles Tendinopathy: Rationale and Implementation. *J Orthop Sports Phys Ther.* 2015;45(11):876-886.

98. Silbernagel KG, Nilsson-Helander K, Thomee R, Eriksson BI, Karlsson J. A new measurement of heel-rise endurance with the ability to detect functional deficits in patients with Achilles tendon rupture. *Knee Surg Sports Traumatol Arthrosc.* 2010;18(2):258-264.

99. Smart GW, Taunton JE, Clement DB. Achilles tendon disorders in runners--a review. *Med Sci Sports Exerc.* 1980;12(4):231-243.

100. Sode J, Obel N, Hallas J, Lassen A. Use of fluroquinolone and risk of Achilles tendon rupture: a population-based cohort study. *Eur J Clin Pharmacol.* 2007;63(5):499-503.

101. Tempfer H, Traweger A. Tendon Vasculature in Health and Disease. *Front Physiol.* 2015;6:330.

102. Theobald P, Benjamin M, Nokes L, Pugh N. Review of the vascularisation of the human Achilles tendon. *Injury.* 2005;36(11):1267-1272.

103. Thornton GM, Hart DA. The interface of mechanical loading and biological variables as they pertain to the development of tendinosis. *J Musculoskelet Neuronal Interact.* 2011;11(2):94-105.

104. Tol JL, Spiezia F, Maffulli N. Neovascularization in Achilles tendinopathy: have we been chasing a red herring? *Knee Surg Sports Traumatol Arthrosc.* 2012;20(10):1891-1894.

105. Tu P. Heel Pain: Diagnosis and Management. *Am Fam Physician.* 2018;97(2):86-93.

106. Uquillas CA, Guss MS, Ryan DJ, Jazrawi LM, Strauss EJ. Everything Achilles: Knowledge Update and Current Concepts in Management: AAOS Exhibit Selection. *J Bone Joint Surg Am.* 2015;97(14):1187-1195.

107. van Mechelen W, Hlobil H, Kemper HC. Incidence, severity, aetiology and prevention of sports injuries. A review of concepts. *Sports Med.* 1992;14(2):82-99.

108. van Schie HT, de Vos RJ, de Jonge S, et al. Ultrasonographic tissue characterisation of human Achilles tendons: quantification of tendon structure through a novel non-invasive approach. *Br J Sports Med.* 2010;44(16):1153-1159.

109. Ward ER, Andersson G, Backman LJ, Gaida JE. Fat pads adjacent to tendinopathy: more than a coincidence? *Br J Sports Med.* 2016;50(24):1491-1492.

110. Wiell C, Szkudlarek M, Hasselquist M, et al. Power Doppler ultrasonography of painful Achilles tendons and entheses in patients with and without spondyloarthropathy: a comparison with clinical examination and contrast-enhanced MRI. *Clin Rheumatol.* 2013;32(3):301-308.

111. Williams JG. Achilles tendon lesions in sport. *Sports Med.* 1986;3(2):114-135.

112. Wise BL, Peloquin C, Choi H, Lane NE, Zhang Y. Impact of age, sex, obesity, and steroid use on quinolone-associated tendon disorders. *Am J Med.* 2012;125(12):1228 e1223-1228 e1228.

113. Wyndow N, Cowan SM, Wrigley TV, Crossley KM. Neuromotor control of the lower limb in Achilles tendinopathy: implications for foot orthotic therapy. *Sports Med.* 2010;40(9):715-727.

CHAPTER I

What does normal tendon structure look like? New insights into tissue characterisation in the Achilles tendon

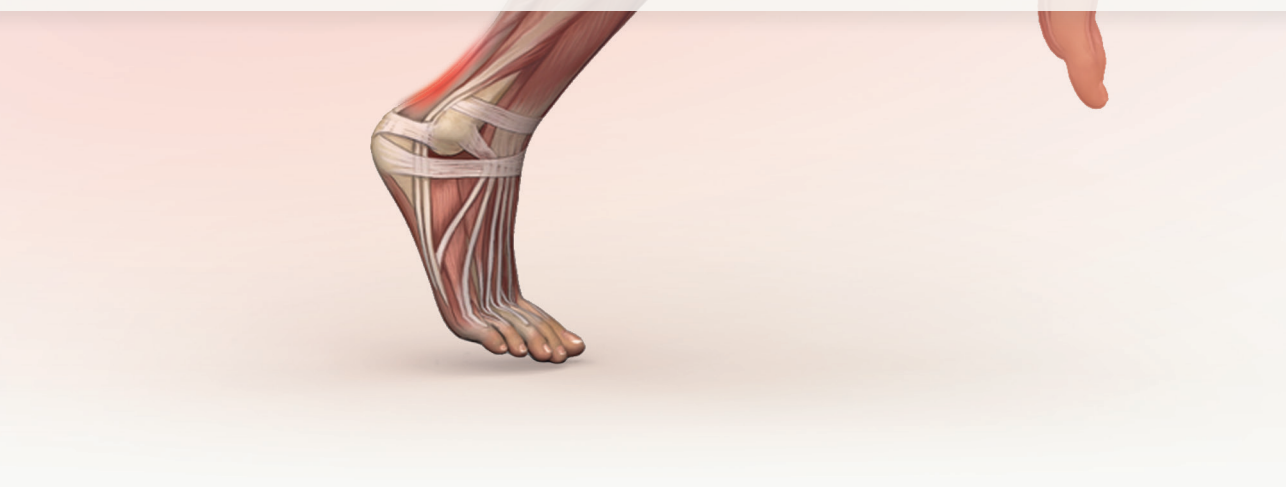
Normal tendon tissue characterisation

E. Wezenbeek[1], N. Mahieu[1], TM. Willems[1,2], D. Van Tiggelen[1,3], M. De Muynck[4], D. De Clercq[5], E. Witvrouw[1,6]

[1] Department of Rehabilitation Sciences and Physiotherapy, Ghent University, Ghent, Belgium, [2] Department of Physical Therapy and Orthopedics, Ghent University, Ghent, Belgium, [3] Department of Physical Medicine & Rehabilitation, Military Hospital of Base Queen Astrid, Belgian Defence, Brussels, Belgium, [4] Department of Physical and Rehabilitation Medicine, Ghent University Hospital, Ghent, Belgium, [5] Department of Movement and Sport Sciences, Ghent University, Ghent, Belgium, [6] Department of Physiotherapy, Aspetar, Doha, Qatar

Accepted for publication in Scandinavian Journal of Medicine & Science in Sports, 2016
Original article (SCI 3.331, Q1) Ranking: 9/81 (Sport Sciences)

Full reference:
What does normal tendon structure look like? New insights into tissue characterization in the Achilles tendon. Wezenbeek E, Mahieu N, Willems TM, Van Tiggelen D, De Muynck M, De Clercq D, Witvrouw E. Scand J Med Sci Sports. 2017 Jul;27(7):746-753.



Abstract

Recently Ultrasound Tissue Characterisation (UTC) was introduced as a reliable method for quantification of tendon structure. Despite increasing publications on the use of UTC, it is striking that there is a lack of normative data in active adolescents. Therefore, the aim of this study was to provide normative values of the Achilles tendon as quantified by UTC. Seventy physiotherapy students (26 male and 44 female) with no history of Achilles tendon injuries were recruited. The Achilles tendons were scanned with UTC to characterise tendon structure. This study demonstrated that Achilles tendons of active, healthy adolescents contained 54.6% echo-type I, 42.8% echo-type II, 2.2% echo-type III, and 0.3% echo-type IV at midportion. The comparison between insertion and midportion of the tendon showed more echo-type II at insertion ($p<0.001$). Furthermore, female tendons contained significantly more echo-type II, in both insertion and midportion compared to male tendons ($p=0.004$ and $p=0.003$, respectively). The results of this study, with respect to the MDC (minimum detectable change), highlight differences in the UTC echopattern in the normal population (sex and regional location), which are important considerations for future studies.

Key words: Achilles tendon, ultrasound, Ultrasound Tissue Characterisation, adolescents, echo-type distribution

Introduction

Although the Achilles tendon is the largest and strongest tendon in the human body, injuries are common.[29] Achilles tendon injuries are frequently found in athletes who participate in racquet sports, track and field, volleyball, soccer and other ball games.[22, 28] The incidence rate can vary between different sports and has been described at 8-9% in rugby players, bicyclists, and runners.[21, 32, 37] The most common Achilles tendon injury is tendinopathy.[1] Clinical signs of tendinopathy are pain, swelling, and loss of function.[6] Despite the high prevalence and the often deleterious consequences of Achilles tendinopathy, many aspects of the aetiology remain unknown.

Recently a new technique was developed to visualize tendon structure; Ultrasound Tissue Characterisation (UTC).[34] This novel technique has been demonstrated to be suitable to quantify subtle changes in tendon structure and has been used in several recent publications.[10, 11, 14, 15, 30, 33, 34] UTC uses conventional ultrasonography to construct a 3D image of the tendon after capturing 600 transverse images over a 12 cm region.[34] Dedicated algorithms quantify the stability of echopatterns over multiple transverse images. Four echo-types can be discriminated, which reflect the structural integrity of the tendon: (echo-type I) intact and aligned tendon bundles; (echo-type II) discontinuous or waving tendon bundles; (echo-type III) mainly fibrillar tissue; and (echo-type IV) amorphous tissue with mainly cellular components and fluid.[34] Echo-type I corresponds with high stability in grey scale pixels over contiguous images. Where there are increasing degrees of variability in grey scale pixel brightness, the tendon is classified as echo-type II, III or IV.[34] According to prior research, the distribution of echo-types representing alterations in tendon bundles alignment (II, III, and IV) represent inferior tendon quality and potentially pathology.[15, 30, 34] However, it remains unclear whether the presence of echo-type II, indicating disorganized and waving tendon bundles, is a positive/adaptive response to load or a negative/early pathological response from the tendon.

Despite increasing publications on the use of UTC, it is striking that there is a lack of normative data at large scale and that there is no available data in young, active adolescents. Previous studies have all evaluated populations older than 24 years and none of the existing studies are suited to represent normative data since the maximum number of participants in these studies was 26, as shown in Table 1. Furthermore, it remains unknown whether sex, dominance or specific location on the Achilles tendon affect the echo-type distribution. This normal tendon characterisation is needed to be able to correctly analyze all future results of UTC studies.

Therefore, the main aim of this study was to investigate the normal tendon characterisation and to describe the distribution of echo-types amongst young, healthy adolescents and this in a large sample. Since there are many indications that underlying degeneration starts long before the onset of symptoms, it might be possible that the tendons of an elder asymptomatic population cannot be regarded per se as normal tendons.[19] Therefore, a young active asymptomatic population was targeted to investigate normal tendon characterisation and to exclude age-related degeneration. Furthermore, this study aimed to assess differences in echo-type distribution between the insertion and the midportion of the tendon and investigate sex-related and dominant-related differences.

Table 1 Overview of normal Achilles tendons imaged in the different studies.

Authors (year of publication)	Number of participants (♂/♀)	Mean age (y)
de Jonge et al[10]	24	46.6
Docking et al[15]	6 (5/1)	26.8
Docking et al[14]	15 (15/0)	23.8
Rosengarten et al[30]	12 (12/0)	23.8
Van Schie et al[34]	26 (16/10)	43.6

Materials and methods

Participants

Seventy freshmen physiotherapy students (29 male and 41 female) at Ghent University, Belgium, participated in this study. Participants were excluded from this study if they had a history of Achilles tendon injuries, a surgery of the lower extremity or pain, ache or soreness in the lower extremity within the previous year. UTC findings of hypoechoic (focal structural change) areas did not lead to exclusion. These 70 students were evaluated during the academic year 2013-2014. All subjects signed informed consent and knew the goals of the study. Mean age of these students was 17.9$\pm$0.5 years (table 2). The average weekly sports participation (basic sports education, practice hours, sports participation outside the official educational programme) was registered and shown in Table 2.

Table 2 Demographics of participants (n = 70)

Age, mean ± SD (y)	17.9 ± 0.5
BMI, mean ± SD	21.0 ± 2.1
Sex	
Male, n (%)	29 (41.4)
Female, n (%)	41 (58.6)
Limb dominance	
Right dominant, n (%)	62 (88.6)
Left dominant, n (%)	8 (11.4)
Time of sports participation, mean ± SD (hours/week)	5.0 ± 3.3

Study design

In all subjects, ultrasound tissue characterisation of both Achilles tendons was performed. Participants were instructed not to engage any sports activities 48 h prior to testing to exclude short-term load-induced changes in the UTC echopattern.[30] The students were asked to fill in a questionnaire concerning demographic and anthropometric data (age, sex, height, weight, limb dominance), sport activities, and injury history. This study was approved by the Ethics Committee of the Ghent University Hospital (number of approval: EC/2013/616).

Ultrasound Tissue Characterisation (UTC)

The UTC measurements were performed by the same experienced researcher (first author of this paper). The subjects were placed in a prone position on the examination table with their feet hanging over the edge and with the ankle in approximately 5–10° dorsiflexion, to slightly pretension the Achilles tendon.[10] A 12 MHz linear-array transducer (Smartprobe 12L5, Terason 2000+, Teratech, Burlington, Massachusetts, USA) moved automatically over the length of the tendon by means of a motor-drive, allowing for transducer tilt, gain, focus, and depth to be standardized (12MHz, focus = 1.3 cm, depth = 3cm). Transverse images were collected at even distances of 0.2 mm and compilation of these images resulted in a 3D data volume block. Tissue characterisation was based on the degree of stability of echopatterns over contiguous images, analyzed by means of dedicated UTC algorithms (UTC 2014, UTC Imaging, Stein, the Netherlands). Four valid echo-types can be discriminated: echo-type I represents intact, continuous and aligned fibers and fasciculi, echo-type II represents less continuous and/or more wavy fibers and fasciculi, echo-type III represents a mainly fibrillar matrix and echo-type IV represents complete disintegration, with tendon tissue replaced by an

amorphous matrix and fluid.[34] The tendons ultra-structure was characterised and quantified by means of relative percentages of these 4 echo-types.

Data analysis

For analysis, Achilles tendons were subdivided into an insertional volume (0-2 cm proximal of the calcaneus) and a midportional volume (2-6 cm proximal of the calcaneus), starting from the proximal border of the calcaneus in proximal direction. Images were analyzed by selecting a region of interest (ROI), defined by the outline of the Achilles tendon in the transverse plane. An example of these so called contours is shown in Figure 1. Five ROIs for the insertion and nine ROIs for the midportion were placed in the longitudinal plane at regular intervals of 5 mm. Contours were then interpolated between these contiguous ROIs creating a tendon volume of the insertion (0-2 cm) and midportion (2-6 cm) separately, in which the proportions of echo-types were quantified. The window size used for interpolation was 17, i.e. tendon structure was quantified with dedicated UTC algorithms that assessed the echopattern by means of relative intensity and distribution of grey levels of corresponding pixels over 17 images (3.2 mm). Each ROI was subdivided into four quadrants; namely posterolateral, anterolateral, anteromedial, and posteromedial. One insertion tendon volume and five midportion tendon volumes were excluded for final analysis, due to possible artifacts.

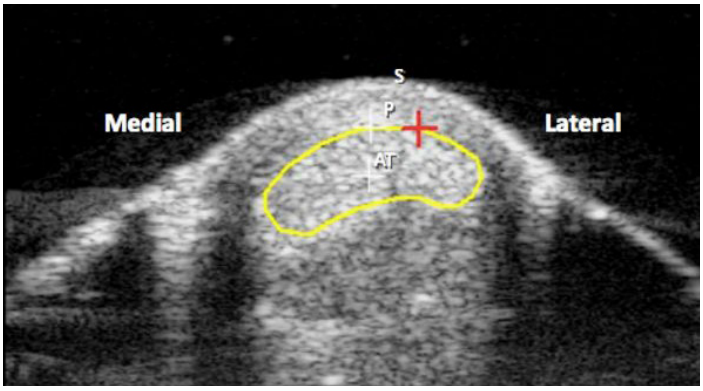


Fig. 1 UTC software. Visualisation of a right AT in the transversal view. Example of a contour made at the ROI in the AT. (S: skin; P: peritendinous space; AT: Achilles tendon.)

Statistical analysis

Differences in echo-types between insertion and midportion, dominant and non-dominant side, medial and lateral, and anterior and posterior part of the tendon were analyzed using the non-parametric Wilcoxon test, as the data were not normally distributed. To investigate differences in echo-types between men and women, a Mann-Whitney U-test was applied. The dominant Achilles tendon was selected for statistical analysis, except for the comparison of the dominant and non-dominant side. Data analysis was done with the SPSS V.22 Statistical Software package (IBM Corp., New York, USA), and the level of significance was set at α=0.05. Test-retest reliability was investigated in ten Achilles tendons and tendon structure was quantified using the same methods as described above. A two-way mixed single measures intra-class correlation for absolute agreement was performed to calculate the standard error of the measurement (SEM= SD of the population x $\sqrt{}$(1-ICC)). The minimal detectable changes (MDC = 1.96 x SEM x $\sqrt{}$2) were calculated.

Results

The Intra Class Correlation Coefficients (ICCs) for intra observer reliability overall were between 0.945 and 0.994, with mean differences between the two analyses performed on the same scan with a time interval of 8 months between -1.25% and 2.27%. The minimal detectable changes were calculated as 5.5%, 5.4%, 0.3% and 0.0% at insertion and 7.0%, 6.5%, 0.8% and 0.2% at midportion for the four echo-types, respectively.

Distribution of UTC echo-types at the insertion and midportion of the AT

Results of the comparison of echo-types between insertion and midportion are visualized in Figure 2. The insertion of the Achilles tendon showed significant differences in echopattern in comparison to the midportion of the tendon. Interestingly, the insertion had significantly more echo-type II tendon bundles ($p<0.001$), whereas more echo-type I were seen in the midportion of the tendon ($p<0.001$). The midportion also contained significantly more echo-types III and IV ($p=0.003$ and $p<0.001$, respectively, table 3). Differences in the echo-types were greater than the minimum detectable change, except for echo-types III and IV.

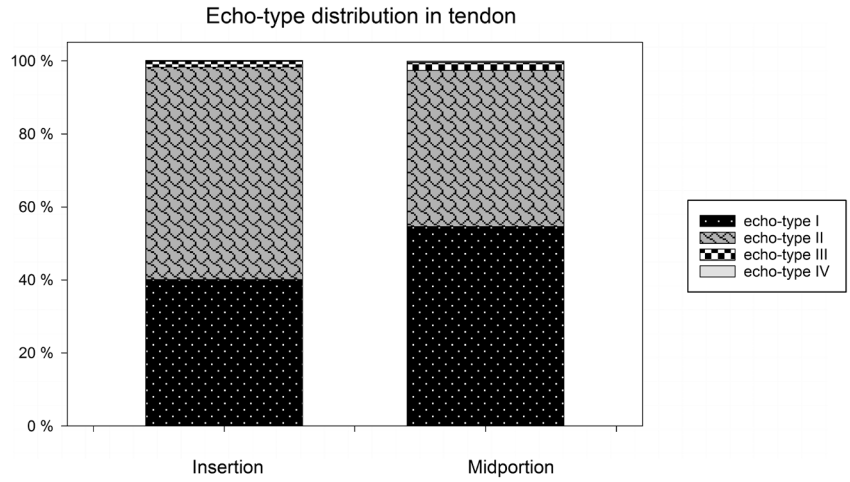


Fig. 2 Distribution of echo-types at the insertion and the midportion of the Achilles tendon.

Table 3 Mean percentages (SD) of UTC echo-types at the insertion and midportion of the Achilles tendon.

	Insertion N = 69	Midportion N = 65	P-value
Echo-type I (%)	40.1 (12.3)	54.6 (14.4)	P<0.001**
Echo-type II (%)	58.2 (10.9)	42.8 (12.3)	P<0.001**
Echo-type III (%)	1.6 (2.8)	2.2 (3.8)	P=0.003*
Echo-type IV (%)	0.2 (0.3)	0.3 (0.5)	P<0.001*

** indicates p<0.05. ** indicates p<0.05 and the difference is greater than the MDC.*

Distribution of UTC echo-types at the anterior & posterior section of the AT

Table 4 shows the results of the comparison of echo-types between the anterior and the posterior part of the tendon, in both insertion and midportion. Significant differences in the echopattern were observed between the anterior and the posterior part of the insertion. The anterior part of the tendon contained significantly more echo-types III and IV in comparison to the posterior part of the tendon

(p<0.001 and p<0.001, respectively), and these differences in echo-types were greater than the MDC. These significant differences in echo-types III and IV were also observed in the midportion of the tendon (p=0.012 and p<0.001, respectively). Furthermore, echo-types I and II also differed significantly in the midportion with anteriorly more type II, and less type I (p=0.025 and p=0.007, respectively). However, these differences in echo-types were not greater than the MDC.

Table 4 Mean percentages (SD) of UTC echo-types at the anterior and posterior section of the Achilles tendon for the insertion and midportion separately.

	Insertion N = 69		P-value	Midportion N = 65		P-value
	Anterior	Posterior		Anterior	Posterior	
Echo-type I (%)	40.2 (10.1)	39.9 (14.4)	P=0.786	53.5 (14.4)	55.9 (15.1)	P=0.007*
Echo-type II (%)	57.6 (8.7)	58.7 (13.2)	P=0.297	43.7 (11.8)	42.0 (13.5)	P=0.025*
Echo-type III (%)	2.0 (3.6)	1.3 (2.5)	P<0.001**	2.5 (4.9)	1.8 (3.2)	P=0.012*
Echo-type IV (%)	0.2 (0.4)	0.1 (0.2)	P<0.001**	0.4 (0.6)	0.2 (0.4)	P<0.001*

** indicates p<0.05. ** indicates p<0.05 and the difference is greater than the MDC.*

Distribution of the UTC echo-types at the medial and lateral section of the AT

Results of the comparison of echo-types between medial and lateral part of the tendon are displayed in table 5. For the insertion as well as for the midportion, a significant difference was seen for type I and II. The lateral side contained more type I (p=0.012 and p=0.024, respectively), whereas the medial side contained more type II (p=0.003 and p=0.023, respectively). Echo-type III was only significantly different at insertion, with more type III in the lateral part of the tendon (p=0.044). Echo-type IV was also significantly more present in the lateral part of the tendon, and this in both insertion and midportion (p=0.020 and p=0.002, respectively). However, these differences in echo-types were not greater than the MDC, except for the difference in echo-type IV at the insertion.

Table 5 Mean percentages (SD) of UTC echo-types at the medial and lateral section of the Achilles tendon.

	Insertion N = 69		P-value	Midportion N = 65		P-value
	Medial	Lateral		Medial	Lateral	
Echo-type I (%)	38.7 (12.0)	41.5 (13.4)	P=0.012*	53.6 (15.6)	55.8 (14.5)	P=0.024*
Echo-type II (%)	59.7 (10.7)	56.6 (12.1)	P=0.003*	44.0 (13.1)	41.7 (12.9)	P=0.023*
Echo-type III (%)	1.5 (2.7)	1.8 (3.1)	P=0.044*	2.2 (4.5)	2.2 (3.6)	P=0.150
Echo-type IV (%)	0.1 (0.3)	0.2 (0.4)	P=0.020**	0.3 (0.4)	0.3 (0.5)	P=0.002*

** indicates p<0.05. ** indicates p<0.05 and the difference is greater than the MDC.*

Difference in distribution of UTC echo-types between male and female

Table 6 presents the difference in echo-types between male and female tendons. The tendons of young, healthy men contained more type I in both the insertion and the midportion (p=0.022 and p=0.008, respectively). In contrast, the tendons of the female population had significantly more type II in both the insertion and the midportion (p=0.004 and p=0.003, respectively). At the insertion, men had significantly more echo-type IV (p=0.037). All these significant differences in echo-types were greater than the MDC. Multiple linear regressions were used to investigate whether physical activity of the participants accounted for significant differences in echopattern between male and female tendons. However, no effects of physical activity were observed.

Difference in distribution of UTC echo-types between the dominant and non-dominant side

No significant differences could be found between the dominant and the non-dominant Achilles tendon, as presented in table 7. This applied for both insertion and midportion.

Table 6 Mean percentages (SD) of UTC echo-types for men and women separately.

Sex	Insertion N = 69		P-value	Midportion N = 65		P-value
	♂ N = 29	♀ N = 40		♂ N = 26	♀ N = 39	
Echo-type I (%)	44.4 (12.2)	37.4 (12.8)	P=0.022**	61.0 (9.4)	50.7 (15.7)	P=0.008**
Echo-type II (%)	53.5 (10.5)	61.0 (11.5)	P=0.004**	37.0 (8.6)	46.5 (13.0)	P=0.003**
Echo-type III (%)	1.8 (3.2)	1.4 (2.3)	P=0.349	1.6 (2.1)	2.6 (4.5)	P=0.688
Echo-type IV (%)	0.2 (0.4)	0.1 (0.2)	P=0.037**	0.4 (0.5)	0.3 (0.5)	P=0.057

*** indicates p<0.05 and the difference is greater than the MDC.*

Table 7 Mean percentages (SD) of UTC echo-types in the dominant and non-dominant Achilles tendon.

Limb dominance	Insertion		P-value	Midportion		P-value
	Non-dominant N = 67	Dominant N = 69		Non-Dominant N = 70	Dominant N = 65	
Echo-type I (%)	42.1 (11.7)	40.1 (12.3)	P=0.214	56.4 (13.8)	54.6 (14.4)	P=0.375
Echo-type II (%)	56.3 (10.5)	58.2 (10.9)	P=0.170	40.7 (11.1)	42.8 (12.3)	P=0.201
Echo-type III (%)	1.5 (3.1)	1.6 (2.8)	P=0.666	2.6 (4.6)	2.2 (3.8)	P=0.602
Echo-type IV (%)	0.2 (0.4)	0.2 (0.3)	P=0.798	0.4 (0.6)	0.3 (0.5)	P=0.400

Discussion

The most striking finding in this study was the high percentage (43- 58%) of echo-type II tendon bundles in the Achilles tendons of active, healthy adolescents since previous research stated that the distribution of echo-types representing alterations in tendon bundles alignment (II, III and IV) possibly represent inferior tendon quality and potentially pathology.[15, 30, 34] Moreover, this study revealed that the tendons of young, healthy men contained more type I in contrast to the female tendons that showed significantly more type II at both the insertion and the midportion. In addition, this study showed some remarkable differences between

insertion and midportion, with the insertion containing more echo-type II tendon bundles, whereas more echo-type I bundles were seen in the midportion of the tendon. Significant differences were also observed in the echo pattern when comparing different regions of the transverse plane as well. The distribution of echo-types I and II differed in the midportion with anteriorly more type II and less type I compared to posterior. Next, the lateral side contained more type I, whereas the medial side contained more type II and this throughout the tendon.

In this study we observed a fairly high percentage of echo-type II fibers (43- 58%) in active, healthy adolescents. This finding is surprising since one would expect that healthy tendons are supposed to be composed of almost solely type I echo fibers, since according to van Schie et al[34] they reflect intact, continuous and aligned fibers and fasciculi, whereas echo-type II fibers are by definition less continuous tendon bundles and represent slight separation and increased waviness of tendon fibrils. In addition, Rosengarten et al[30] writes that a decrease in echo-type I coinciding an increase in echo-type II suggests that the normal tendon integrity has been negatively affected. In this study the subjects were adolescent athletes. This could be a possible explanation for observing such a high percentage of echo type II fibers. One important consideration when dealing with adolescent athletes is the consequences of maturation.[2] Previous research demonstrated that maturation affects the properties of muscle and tendon.[26] Heinemeier et al[18] measured ^{14}C content within the tendon and concluded that tissue turnover is mostly seen during adolescence, indicating tendon adaptability, and is limited hereafter. Rosengarten et al[30] stated that echo-type II is indicative for reversible tendon matrix remodeling, leading to the consideration that the higher percentage echo-type II in our young, active population could possibly be explained by the higher tissue turnover during adolescence. Since this study is the first to examine the normal tendon characterisation in a healthy active adolescent population, comparison with other studies is rather difficult. Furthermore, the number of studies using UTC is still small and previous studies have used different scanning positions (weight-bearing versus non-weight-bearing), a smaller sample size, and showed differences in the analyzed tendon volume.[9, 12, 14, 15, 30, 34] These differences indicate that caution is advised in comparing UTC data from previous studies to our results. However, looking at the results of the previous studies, our study showed a higher percentage echo-type II than any of the other published studies using UTC in a healthy population.[12, 14, 15, 30, 34] Rosengarten et al[30] found that the tendon was composed of 92% of echo-type I. In the study of van Schie et al[34] approximately the same amount of type I (48%), but less type II (28%), and more type III (13%) and IV (11%) tendon bundles were detected. This variation in echo-types could partially be explained by the study population. The participants in this study were both male

and female freshmen students, with a mean age of 18 years. Interestingly, previous studies have all evaluated populations older than 24 years.[10, 11, 14, 15, 30, 34] Mean age of the participants in the study of van Schie et al[34] was 45 years. Previous research stated that age is commonly associated with increased prevalence of degenerative changes, such as decreased cellularity, increased glycosaminoglycan content and lack of fiber organization.[23] Therefore, it might be possible that the tendon of an elder population shows differences in echopattern compared to the adolescent tendons observed in this study. Further research is necessary to elucidate this. Furthermore, both Docking et al[15] and Rosengarten et al[30] evaluated exclusively male elite football players (mean age 24 years), to examine load-related tendon change. Docking et al[15] found a significant increase in echo-type I after a 5-month training period, suggesting that the UTC echopattern improves due to high loads, which might explain the high amount of echo-type I present in their athlete population. Besides the fact that our sample also included women, meaning more echo-type II, our population was also younger. As mentioned before, an important explanation for the observed difference in echo-types between studies could be due to the use of different UTC components, protocols and analyzing techniques. For example, Docking et al[15], Rosengarten et al[30], and van Ark et al[33] used window size 25 (consecutive images correlated over 4.8mm), whereas this study analyzed with window size 17 (consecutive images correlated over 3.2mm). A smaller window size means that images can be analyzed in greater detail. This is more suited for detection of small-scale waviness, therefore our results are probably a better representation of the actual tendon structure.

This study is the first to report the comparison in echo-type distribution between the insertion and midportion of the tendon. The results of this study identified that the insertion of the Achilles tendon contained more echo-type II, whereas echo-type I was more present at midportion. As stated by van Schie et al[34] echo-type II is a representation of an elevated percentage of wavy tendon bundles. The shape of the Achilles tendon varies from proximal to distal as it approaches its calcaneal attachment site.[3] Flaring of the Achilles tendon at calcaneal attachment is an adaptation to securing skeletal anchorage.[4] More proximally, especially at midportion, the tendon becomes more cylindrical in shape with linear arrangement of fibers, suggesting internal reorganization of Achilles tendon fascicles.[4, 27] These morphological observations explain the high presence of echo-type II at insertion compared to midportion. Another possible explanation for this difference between the insertion and midportion might be compositional differences. A immunohisto-chemical study demonstrated striking differences in the extracellular matrix (ECM) between the Achilles tendon midportion and its insertion.[36] The range and distribution of ECM molecules detected in the Achilles tendon reflect the differing

forces acting on it; the midportion largely transmits tension and is characterised by molecules typical of fibrous tissues, whereas the insertion must also resist compression and thus also contain fibrocartilages molecules, like aggrecan.[36] Aggrecan is a proteoglycan (PG) that exhibits a bottlebrush structure, in which glycosaminoglycan (GAG) chains are attached to an extended protein core.[25] The localized high concentrations of aggrecan provide the osmotic properties necessary for normal tissue function with the GAGs producing the swelling pressure that counters compressive loads on the tissue.[31] Importantly, recent studies have shown that the fibrillogenesis of the tendon is regulated by PGs, associated with aggrecan, that are present in the ECM.[8, 13, 16] Since PGs regulate the assembly of the chief structural component of the tendon, this might explain the structural differences found in the insertion compared to the midportion of the Achilles tendon. In support of these notions the presence of echo-type II may not indicate inferior tendon quality but a necessary morphological, histological and functional requirement.

When comparing different compartments of the tendon, significant differences between both the anterior and posterior part as well as between the medial and lateral part of tendon were found, although these significant differences were not greater than the MDC. At the midportion, the anterior part of the tendon contained a greater amount of echo-type II and to a lesser extent echo-type I compared to the posterior part. Furthermore, when medial and lateral sections were compared, the lateral side contained more type I, whereas the medial side contained more type II. Recently, van Sterkenburg et al[35] described the hypothesis of compression between the Achilles tendon and the M. Plantaris tendon, which is located medially to the Achilles tendon. The M. Plantaris tendon is stiffer, less extensible, and could create repeated shear stresses to the Achilles tendon.[20] This involvement of the M. Plantaris might explain the increased presence of echo-type II, i.e. waving tendon bundles, at the medial side of the Achilles tendon. Interestingly, these locations are more frequently involved in tendinopathy compared to the posterior and lateral midportion of the tendon.[7, 17]

Finally, another interesting finding in this study showed that the tendons of young, healthy men contained more echo-type I in contrast to the tendons of the female population, who showed significantly more type II. Moreover, linear regression analyses showed that physical activity of the participants did not account for these significant differences observed in echopattern between male and female tendons. This study demonstrates a difference in UTC echo-type between female and male participants that has not previously been identified. However, previous studies stated that there are sex-related differences in the mechanical properties of the

tendon fascicles.[24] Furthermore, Bryant et al[5] found that estrogen has an effect on both the functional and structural properties of the Achilles tendon, which might explain this sex difference in echo-types distribution.

The results of this study have important clinical implications since, in line with our findings, we suggest that the high percentage echo-type II (42.8%) tendon bundles is a normal physiological finding in an active, adolescent population and should not be considered as abnormal. Furthermore, our results also suggest to analyze male and female data differently since a sex difference in echo-types distribution was found, with more echo-type II in female tendons. Moreover, the finding of more echo-type II at the insertion, compared to the midportion should be taken into account when evaluating UTC results. Therefore, our large-scale data highlights important differences, with respect to the MDC, in the UTC echopattern in the normal population which needs to be considered in future studies.

A limitation of this study is that our results concern an active, adolescent population. Therefore, these results cannot be generalized. Nevertheless, this population was targeted to exclude age-related degeneration and represents normal, healthy tendons. Another limitation of this study is that it was performed in an age-cohort that does not get Achilles tendinopathy. This may limit comparison of the pathological tendon in future studies, as the ages are likely to be significantly different. Also, no US imaging of the tendon structure was performed to exclude/include subjects in this study. The strength of this study is that this is the first study to report UTC findings on a large sample size compared to previous studies.[10, 11, 14, 15, 30, 33, 34]

Perspectives

This study was the first to investigate the normal tendon characterisation and this is the largest observational study of normal tendons. UTC has been demonstrated to be suitable to quantify subtle differences in tendon structure that may precede the development of symptoms.[34] However, before establishing aberrant tendon structure, normative data are imperative and differences between sexes, and different locations in the tendon should be taken into account.

References

1. Asplund CA, Best TM. Achilles tendon disorders. *BMJ.* 2013;346:f1262.
2. Barker AR, Armstrong N. Exercise testing elite young athletes. *Med Sport Sci.* 2011;56:106-125.
3. Benjamin M, Kaiser E, Milz S. Structure-function relationships in tendons: a review. *J Anat.* 2008; 212(3):211-228.
4. Benjamin M, Toumi H, Ralphs JR, Bydder G, Best TM, Milz S. Where tendons and ligaments meet bone: attachment sites ('entheses') in relation to exercise and/or mechanical load. *J Anat.* 2006;208(4):471-490.
5. Bryant AL, Clark RA, Bartold S, et al. Effects of estrogen on the mechanical behavior of the human Achilles tendon in vivo. *J Appl Physiol (1985).* 2008;105(4):1035-1043.
6. Cook JL, Purdam CR. Is tendon pathology a continuum? A pathology model to explain the clinical presentation of load-induced tendinopathy. *Br J Sports Med.* 2009;43(6):409-416.
7. Counsel P, Comin J, Davenport M, Connell D. Pattern of Fascicular Involvement in Midportion Achilles Tendinopathy at Ultrasound. *Sports Health.* 2015;7(5):424-428.
8. Danielson KG, Baribault H, Holmes DF, Graham H, Kadler KE, Iozzo RV. Targeted disruption of decorin leads to abnormal collagen fibril morphology and skin fragility. *J Cell Biol.* 1997;136(3):729-743.
9. de Jonge S, de Vos RJ, Weir A, et al. One-year follow-up of platelet-rich plasma treatment in chronic Achilles tendinopathy: a double-blind randomized placebo-controlled trial. *Am J Sports Med.* 2011;39(8):1623-1629.
10. de Jonge S, Rozenberg R, Vieyra B, et al. Achilles tendons in people with type 2 diabetes show mildly compromised structure: an ultrasound tissue characterisation study. *Br J Sports Med.* 2015;49(15):995-999.
11. de Vos RJ, Heijboer MP, Weinans H, Verhaar JA, van Schie JT. Tendon structure's lack of relation to clinical outcome after eccentric exercises in chronic midportion Achilles tendinopathy. *J Sport Rehabil.* 2012;21(1):34-43.
12. de Vos RJ, Weir A, Tol JL, Verhaar JA, Weinans H, van Schie HT. No effects of PRP on ultrasonographic tendon structure and neovascularisation in chronic midportion Achilles tendinopathy. *Br J Sports Med.* 2011;45(5):387-392.
13. Derwin KA, Soslowsky LJ, Kimura JH, Plaas AH. Proteoglycans and glycosaminoglycan fine structure in the mouse tail tendon fascicle. *J Orthop Res.* 2001;19(2):269-277.
14. Docking SI, Rosengarten SD, Cook J. Achilles tendon structure improves on UTC imaging over a 5-month pre-season in elite Australian football players. *Scand J Med Sci Sports.* 2016;26(5):557-563.
15. Docking SI, Rosengarten SD, Daffy J, Cook J. Structural integrity is decreased in both Achilles tendons in people with unilateral Achilles tendinopathy. *J Sci Med Sport.* 2015;18(4):383-387.
16. Ezura Y, Chakravarti S, Oldberg A, Chervoneva I, Birk DE. Differential expression of lumican and fibromodulin regulate collagen fibrillogenesis in developing mouse tendons. *J Cell Biol.* 2000;151(4):779-788.
17. Gibbon WW, Cooper JR, Radcliffe GS. Distribution of sonographically detected tendon abnormalities in patients with a clinical diagnosis of chronic achilles tendinosis. *J Clin Ultrasound.* 2000;28(2):61-66.
18. Heinemeier KM, Schjerling P, Heinemeier J, Magnusson SP, Kjaer M. Lack of tissue renewal in human adult Achilles tendon is revealed by nuclear bomb (14)C. *FASEB J.* 2013;27(5):2074-2079.
19. Hirschmuller A, Frey V, Konstantinidis L, et al. Prognostic value of Achilles tendon Doppler sonography in asymptomatic runners. *Med Sci Sports Exerc.* 2012;44(2):199-205.
20. Lintz F, Higgs A, Millett M, et al. The role of Plantaris Longus in Achilles tendinopathy: a biomechanical study. *Foot Ankle Surg.* 2011;17(4):252-255.
21. Lopes AD, Hespanhol Junior LC, Yeung SS, Costa LO. What are the main running-related musculoskeletal injuries? A Systematic Review. *Sports Med.* 2012;42(10):891-905.
22. Maffulli N, Sharma P, Luscombe KL. Achilles tendinopathy: aetiology and management. *J R Soc Med.* 2004;97(10):472-476.
23. Magnan B, Bondi M, Pierantoni S, Samaila E. The pathogenesis of Achilles tendinopathy: a systematic review. *Foot Ankle Surg.* 2014;20(3):154-159.
24. Magnusson SP, Hansen M, Langberg H, et al. The adaptability of tendon to loading differs in men and women. *Int J Exp Pathol.* 2007;88(4):237-240.

25. Nap RJ, Szleifer I. Structure and interactions of aggrecans: statistical thermodynamic approach. *Biophys J.* 2008;95(10):4570-4583.
26. O'Brien TD, Reeves ND, Baltzopoulos V, Jones DA, Maganaris CN. Mechanical properties of the patellar tendon in adults and children. *J Biomech.* 2010;43(6):1190-1195.
27. Obst SJ, Renault JB, Newsham-West R, Barrett RS. Three-dimensional deformation and transverse rotation of the human free Achilles tendon in vivo during isometric plantarflexion contraction. *J Appl Physiol (1985).* 2014;116(4):376-384.
28. Paavola M, Kannus P, Paakkala T, Pasanen M, Jarvinen M. Long-term prognosis of patients with achilles tendinopathy. An observational 8-year follow-up study. *Am J Sports Med.* 2000;28(5):634-642.
29. Ram R, Meeuwisse W, Patel C, Wiseman DA, Wiley JP. The limited effectiveness of a home-based eccentric training for treatment of Achilles tendinopathy. *Clin Invest Med.* 2013;36(4):E197-206.
30. Rosengarten SD, Cook JL, Bryant AL, Cordy JT, Daffy J, Docking SI. Australian football players' Achilles tendons respond to game loads within 2 days: an ultrasound tissue characterisation (UTC) study. *Br J Sports Med.* 2015;49(3):183-187.
31. Roughley P, Martens D, Rantakokko J, Alini M, Mwale F, Antoniou J. The involvement of aggrecan polymorphism in degeneration of human intervertebral disc and articular cartilage. *Eur Cell Mater.* 2006;11:1-7; discussion 7.
32. Sankey RA, Brooks JH, Kemp SP, Haddad FS. The epidemiology of ankle injuries in professional rugby union players. *Am J Sports Med.* 2008;36(12):2415-2424.
33. van Ark M, Docking SI, van den Akker-Scheek I, et al. Does the adolescent patellar tendon respond to 5 days of cumulative load during a volleyball tournament? *Scand J Med Sci Sports.* 2016;26(2):189-196.
34. van Schie HT, de Vos RJ, de Jonge S, et al. Ultrasonographic tissue characterisation of human Achilles tendons: quantification of tendon structure through a novel non-invasive approach. *Br J Sports Med.* 2010;44(16):1153-1159.
35. van Sterkenburg MN, van Dijk CN. Mid-portion Achilles tendinopathy: why painful? An evidence-based philosophy. *Knee Surg Sports Traumatol Arthrosc.* 2011;19(8):1367-1375.
36. Waggett AD, Ralphs JR, Kwan AP, Woodnutt D, Benjamin M. Characterization of collagens and proteoglycans at the insertion of the human Achilles tendon. *Matrix Biol.* 1998;16(8):457-470.
37. Weiss BD. Nontraumatic injuries in amateur long distance bicyclists. *Am J Sports Med.* 1985;13(3):187-192.

CHAPTER II

Is Achilles tendon blood flow related to foot pronation?

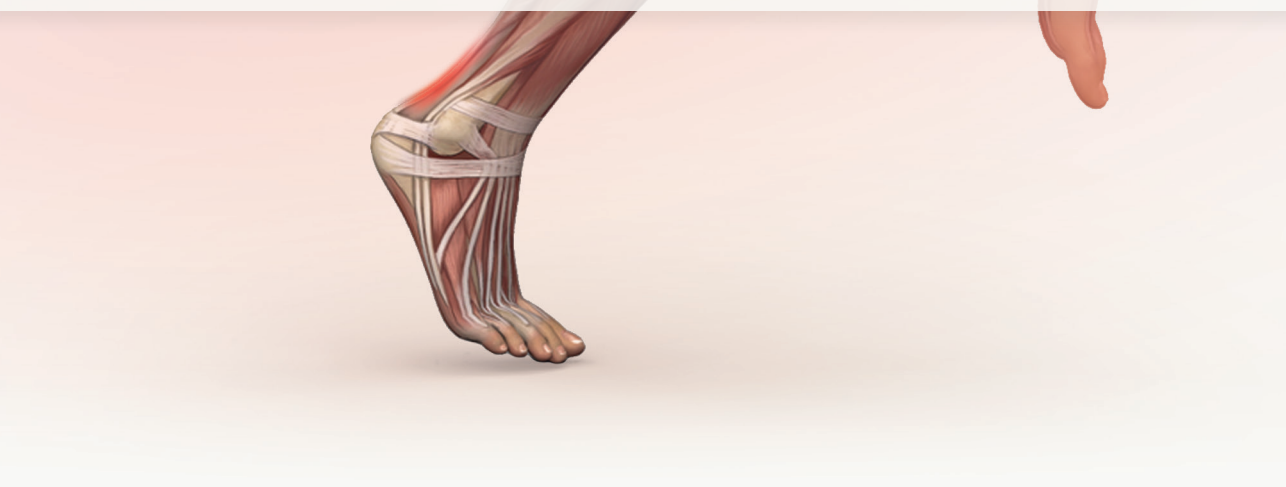
Association between kinematics and blood flow

E. Wezenbeek[1], TM. Willems[1], N. Mahieu[1], I. Van Caekenberghe[2], E. Witvrouw[1], D. De Clercq[3]

[1] Department of Rehabilitation Sciences and Physiotherapy, Ghent University, Ghent, Belgium,
[2] Ine Van Caekenberghe, research consultancy, Sint-Lievens-Houtem, Belgium,
[3] Department of Movement and Sport Sciences, Ghent University, Ghent, Belgium

Accepted for publication in Scandinavian Journal of Medicine & Science in Sports, 2016
Original article (SCI 3.331, Q1) Ranking: 9/81 (Sport Sciences)

Full reference:
Is Achilles tendon blood flow related to foot pronation? Wezenbeek E, Willems TM, Mahieu N, Van Caekenberghe I, Witvrouw E, De Clercq D. Scand J Med Sci Sports. 2017 Dec;27(12):1970-1977.



Abstract

In the etiology of Achilles tendinopathy (AT) it is frequently suggested that excessive pronation causes a vascular constriction of the Achilles tendon, described as the "whipping phenomenon".[6] Although previous studies focused on the association between pronation and AT, it is striking that the underlying association between foot pronation and blood flow has not been studied yet. Therefore, the aim of this study was to investigate whether the amount of pronation during running influences the Achilles tendon blood flow. Twenty-five experienced runners, aged 34.5 $\pm$ 10.2 years, participated in this study. 2D-lower limb kinematics during barefoot and shod running in both frontal and sagittal plane were assessed. Blood flow of the Achilles tendon was measured before and after barefoot and shod running, using the oxygen-to-see device. The results of this study showed a significant effect of eversion excursion on the increase in Achilles tendon blood flow after shod running. More specifically, the more eversion excursion observed, the lower the increase in blood flow ($p = 0.013$). We therefore suggest, in individuals with increased inversion at touch down and increased eversion around midstance during shod running, that anti-pronation measures could be useful in both preventing and managing Achilles tendinopathy.

Key words: Achilles tendon, pronation, whipping phenomenon, eversion, blood flow, vascular constriction, running.

Introduction

The Achilles tendon is the thickest and strongest tendon in the human body. Nevertheless, injuries are common, especially in runners.[3] Epidemiologic studies show that 5-34% of all runners develop Achilles tendon pain or Achilles tendinopathy (AT).[12, 16, 18] Hence, AT is one of the most common overuse injuries for both recreational and elite runners. Numerous studies and reviews have therefore been published investigating intrinsic and extrinsic risk factors for developing AT to better understand the multifactorial mechanism of AT.[13] Abnormalities in movement patterns as a risk factor for AT have been a topic of discussions since the 1980s. In particular, foot pronation is believed to be strongly associated with AT injuries based on several cross-sectional analyses.[6, 7, 20, 27] Pronation is a triplanar motion involving a combination of rearfoot eversion, dorsiflexion and abduction of the foot.[30] It has been suggested that excessive foot pronation creates a whipping or torsional action upon the Achilles tendon, which is defined as the whipping phenomenon, as the foot rotates rapidly from an inverted position at heel strike to an excessively everted position in midstance. Vascular blanching of the midportion of the Achilles tendon is thought to result from this purported torsional effect.[6, 26, 28] This whipping hypothesis is supported by more recent studies, where it is found that subjects with AT averagely demonstrate greater maximum rearfoot eversion (±2.23°) and greater rearfoot eversion range of motion (±4.77°) during running than controls.[7, 20, 27]

Despite evidence linking pronation to the onset of AT, there is little to no conclusive evidence of the injury mechanism behind this. Excessive eversion has been hypothesized to accentuate the rotation of the tendon and cause a wringing out of the tendon, leading to AT. However,previous studies stated that the Achilles tendon blood flow increases during and after activity, in comparison with the measured resting values, and that this increase in blood flow is a normal physiological response to activity.[4, 15] If excessive subtalar joint movement accentuates the torsion of the tendon[6] sufficiently to cause constriction of the vascular networks,[32] then it is possible that more eversion during running may induce a lower increase in blood flow after running. However, although this hypothesis is often described as an injury mechanism to develop AT, it is striking that this has not been studied yet. Therefore, the aim of this study is to investigate whether the amount of eversion during running influences Achilles tendon blood flow.

Materials and methods

This experimental study was performed at the Runners Service Lab, Zwijndrecht, Belgium. Approval was obtained by the Ethics Committee of the Ghent University Hospital (number of approval: EC/2013/984) and all participants signed an informed consent. Lower limb kinematics during running in both the frontal (dorsal view) and the sagittal plane were assessed. Blood flow measurements of the Achilles tendon were performed before running trials, and after barefoot and shod running.

Participants

Twenty-five healthy subjects participated in this study. Participants were included only when their average mileage was at least 30 km a week. Exclusion criteria for this study were: (a) pain, ache or soreness in the lower extremity within the previous year (with specific attention for Achilles tendon pain), (b) surgery of the lower extremity, (c) neurologic problems that would affect lower extremity function. Table 1 presents the characteristics of the participants.

Table 1 Demographics of participants (n = 25).

Age, mean ± SD (y)	34.5 ± 10.2
Weight, mean ± SD (kg)	68.3 ± 13.1
Length, mean ± SD (m)	1.78 ± 0.1
BMI, mean ± SD (kg/m^2)	21.4 ± 2.1
Sex	
Male, n (%)	15 (60)
Female, n (%)	10 (40)
Limb dominance	
Right dominant, n (%)	18 (72)
Left dominant, n (%)	7 (28)
Time of running participation, mean ± SD (hours/week)	6.1 ± 2.4

Study design

Participants were instructed not to engage in any sports activities 48 h prior to testing to ensure a valid measure of exercise related increase in blood flow. First, participants were instructed to rest on a treatment table for 10 minutes. After this resting period, blood flow was measured for a first time. The blood flow was measured approximately for 10 seconds per measurement point, in the same prone position on this treatment table. This blood flow measurement was expressed as

initial tendon blood flow. Then, prior to the actual running tasks, marker placement and a static standing trial (10 sec) was executed. After this 10 minutes of preparation, the actual running task started with 10 minutes of barefoot running at a self-selected speed during which kinematics were recorded. Immediately after this, blood flow measurements were repeated in the same prone position on the treatment table (post-barefoot run blood flow). The treatment table was positioned next to the running track to minimalize the transition time. Next, participants were asked to run shod for 10 minutes wearing standard neutral Pearl Izumi© shoes. Finally, a post-shod running measurement of the blood flow was conducted immediately after the running task (post-shod run blood flow). In conclusion, three blood flow measurements were conducted: initial blood flow, immediate post-barefoot run blood flow, and immediate post-shod run blood flow. Prior to the actual testing procedure, participants tried out the Pearl Izumi© shoes of their size for a smooth transition to the shod running trial. The Pearl Izumi© shoes had a heel-toe offset of 7 mm and no special features to prevent excessive pronation. To guarantee a valid study, this study was performed at Runners Service Lab, a specialized running Lab. The standard protocol of Runners Service Lab was applied, consisting of barefoot running trials followed by shod running trials.

Participants were instructed to run at a self-selected speed during both running conditions (barefoot and shod). In order to keep the self-selected speed as constant as possible, Noptel© (Teknologiantie 2, FI-90590 Oulu, Finland) (CM-distance sensor, type CM5) was used, a laser registration device with a precision of 0.1 $m.s^{-1}$. Participants were asked to correct their speed if there was a deviation of 0.2 $m.s^{-1}$ from their average running speed. Between both running conditions a maximal difference of 0.2 $m.s^{-1}$ was allowed. Average running speed was 3.1 (SD = 0.4) $m.s^{-1}$ during barefoot running and 3.2 (SD = 0.4) $m.s^{-1}$ during the shod condition.

Before the experimental protocol, the participants were asked to fill in a questionnaire concerning demographic and anthropometric data (age, sex, height, weight, limb dominance), running activities, and injury history.

To obtain a reproducible location for the blood flow measurements, bilateral skin markers were placed for transducer placement at the point 4 cm proximal from the upper border of the calcaneal bone in the centre of the Achilles tendon, representing the midportion of the Achilles tendon. Blood flow data were collected by use of the oxygen-to-see® (O2C) device (LEA Medizintechnik, Giessen, Germany). The O2C is a non-invasive device capable of measuring the perfusion and oxygenation of the subcutaneous tissue up to a depth of 8 mm by using an optical fiber probe. The fiber probe incorporates both white light spectroscopy (wavelengths of

500-800 nm) and the laser Doppler technique (830 nm and 30 mW). The white light spectroscopy allows to detect haemoglobin parameters. The laser Doppler flowmetry allows to determine perfusion parameters in the tissue as it detects all moving erythrocytes. The number of moving erythrocytes combined with the blood flow velocity is processed to the parameter blood flow which is expressed in arbitrary units. This device has been shown to be reliable and valid.[9, 11, 33] Environmental factors such as light and temperature were kept constant for standardization purposes. All measurements were performed by the same experienced researcher (first author of this study). The stability of the measurement of blood flow has been investigated for the measurement point 4 cm proximal from the upper border of the calcaneal bone in the center of the Achilles tendon. A two-way mixed single measures intra-class correlation for absolute agreement was performed after a ten min rest period in ten Achilles tendons of age-matched subjects. The ICC for intra observer reliability was 0.75 for this measurement point.

The set-up for the kinematic measurements is illustrated in Figure 1. Participants were instructed to run on a 45 m long Mondo© running track, while 4 synchronized Quintic© GigE High-Speed LIVE cameras (125 Hz) and Quintic© software (Columbiaville, MI) were used for the recording of both the frontal (1 dorsal camera) and sagittal plane (1 left, 2 right cameras) during both running conditions (barefoot and shod).

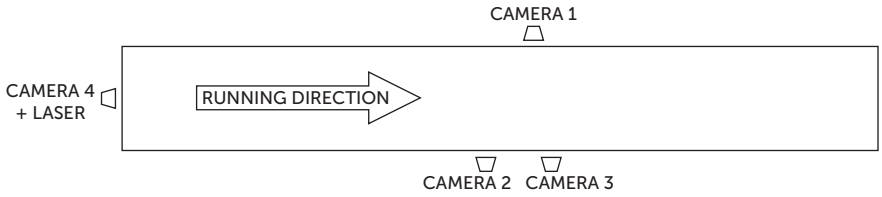


Fig. 1 Study set-up for the kinematic measurements during barefoot and shod running.

Eight reflective 2D-markers were placed on anatomical reference points, as illustrated in Figure 2. Markers were placed bilaterally: 2 markers were placed on the midline of the heel (one distal and one proximal), 2 markers on the Achilles tendon (one distal and one in between the lateral and medial head of the m. Gastrocnemius), and one marker on the lateral aspect of the fifth metatarsal head, on the distal point of lateral malleolus, on the lateral knee joint line, and on the greater trochanter.

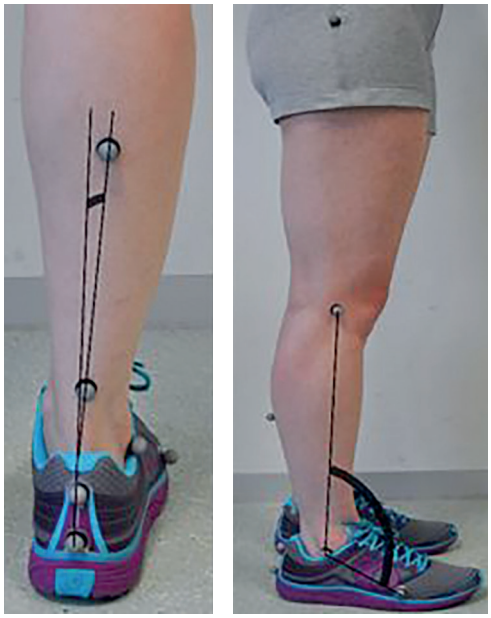


Fig. 2 Right Achilles tendon angle and ankle angle in shod condition.

Data analysis

The reflective markers were digitized using Maxtraq® version 2.6.1.1(©Innovision Systems, Inc., Natick, MA). For each running condition three trials (containing on average 4 stances in dorsal view and 2 stances in sagittal view) for which full sagittal and frontal kinematics could be obtained were randomly selected. For all included variables (Achilles tendon angle and ankle angle, see next paragraph) average values were calculated for both sides of the 25 subjects, based on 12 stances (averagely 4 stances per 3 trials) for the dorsal variables and on 6 stances (averagely 2 stances per 3 trials) for the sagittal variables. Initial contact and toe-off were visually defined as respectively the first and last instant of foot-ground contact. In both sagittal and dorsal views, marker digitisation started 10 frames before initial foot contact and finished 10 frames after toe-off for the sagittal views and 10 frames after heel-off for the dorsal views.

Calculations of kinematic variables were performed in Matlab® (©The MathWorks, Inc., Natick, Massachusetts, United States). 2D-marker trajectories were filtered using a second order Butterworth low-pass filter with a cut-off frequency of 18 Hz with mirrored endpoint extrapolation to avoid endpoint artefacts.

Kinematic variables of interest were the ankle angle in the sagittal plane (plantarflexion-dorsiflexion) defined by the angle between the markers on the lateral aspect of the fifth metatarsal head, on the distal point of lateral malleolus, and on the lateral knee joint line, and also the Achilles tendon angle in the frontal plane (inversion-eversion) defined by markers on the Achilles tendon and the heel markers (Figure 2). Eversion is defined as an inward rotation of the foot with respect to the longitudinal foot axis. For the ankle and Achilles tendon angles, eversion and dorsiflexion excursion was calculated. Eversion excursion is determined as the difference between the Achilles tendon angle at initial contact or maximal initial inversion, if initial contact was not the maximal initial inversion, and the maximal eversion angle (illustrated in Figure 3). Dorsiflexion excursion is determined as the difference between the ankle angle at initial contact or maximal initial plantarflexion, if initial contact was not the maximal initial plantarflexion, and maximal dorsiflexion. Left sides were mirrored to obtain positive eversion and dorsiflexion excursions for both sides. Since it has been suggested that the whipping action upon the Achilles tendon occurs as the foot rotates rapidly from an inverted position at heel strike to an excessively everted position in midstance, velocities were also calculated. Average and maximal dorsiflexion and eversion velocities were calculated using the first time-derivative of the respective angles. Initial foot contact pattern was determined because it influences the following foot-ankle movements during the first part of stance and thus can influence Achilles tendon blood flow.[5] Foot angles at initial contact were calculated taking into account the foot angles during the static measurement, classifying participants in 3 groups: initial rearfoot, midfoot, or forefoot contact pattern.[1]

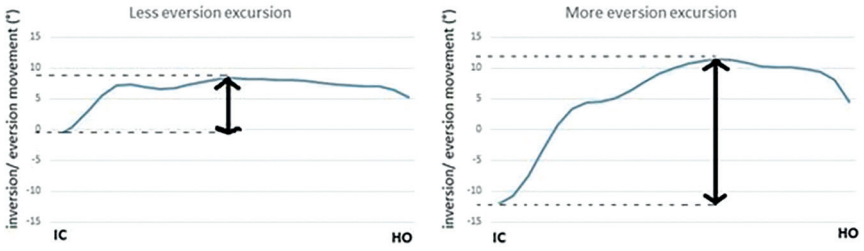


Fig. 3 Examples of the inversion/eversion movement from initial contact (IC) to heel off (HO) from the participants with the least (8°) and the most (24°) eversion excursion.

Statistical analysis

Statistical analysis was done with the SPSS V.23 Statistical Software package (IBM Corp., New York, USA). The effect of kinematic variables (eversion excursion, dorsiflexion excursion, average and maximal velocities, and initial foot strike pattern) on the increase in blood flow after barefoot and shod running (post-barefoot/shod run blood flow minus initial blood flow) was investigated with linear mixed models. Analyses were executed with participants as a random factor, and kinematic variables as fixed predictors. The effect of possible covariates as age, sex, running speed, and limb dominance was investigated. The residuals of the linear mixed model were checked for normal distribution and homoscedasticity. The level of significance was set at α=0.05.

Results

Sex, running speed, age and limb dominance did not have an effect on the outcome parameter, and are therefore not included as covariates in the analyses.

Table 2 displays the descriptive values of the kinematic variables (Achilles tendon and ankle angle) and blood flow variables. A significant difference is seen between both conditions (barefoot and shod) for eversion and dorsiflexion excursion ($p < 0.05$). Furthermore, there was a significant increase in blood flow after barefoot and shod running compared to initial tendon blood flow ($p < 0.05$). The blood flow descriptives show an increase in blood flow of 42.6 % after barefoot running and an increase in blood flow of 61.7% after shod running.

Table 2 Descriptive of kinematic and blood flow variables.

Dorsiflexion excursion barefoot running, mean ± SD (°)	24.6 ± 5.6	⋆
Dorsiflexion excursion shod running, mean ± SD (°)	22.2 ± 3.8	
Eversion excursion barefoot running, mean ± SD (°)	11.0 ± 4.0	⋆
Eversion excursion shod running, mean ± SD (°)	15.6 ± 3.5	
Initial blood flow, mean ± SD (AU)	55.1 ± 25.6	⋆
Blood flow after barefoot running, mean ± SD (AU)	78.6 ± 41.5	⋆
Blood flow after shod running, mean ± SD (AU)	89.1 ± 50.1	

* p-value < 0.05

The kinematic variables did not significantly influence the increase in blood flow after barefoot running. However, univariate analysis showed a significant effect of eversion excursion on the increase in blood flow after shod running (F= 6.68, p= 0.013). The result of the linear mixed model analysis is shown in Table 3. The more eversion excursion during running, the lower the increase in blood flow after shod running. Eversion excursion accounts for 10,6% of the within-participants variability in the increase in blood flow after shod running.

Table 3: Effect of eversion excursion on increase in blood flow after shod running.

	b	SE b	P-value	95% Confidence Interval	
				Lower Bound	Upper Bound
Intercept	102,52	27,33	0,000	47,54	157,49
Eversion excursion	- 4,42	1,71	**0,013***	- 7,86	- 0,98

* p-value < 0.05

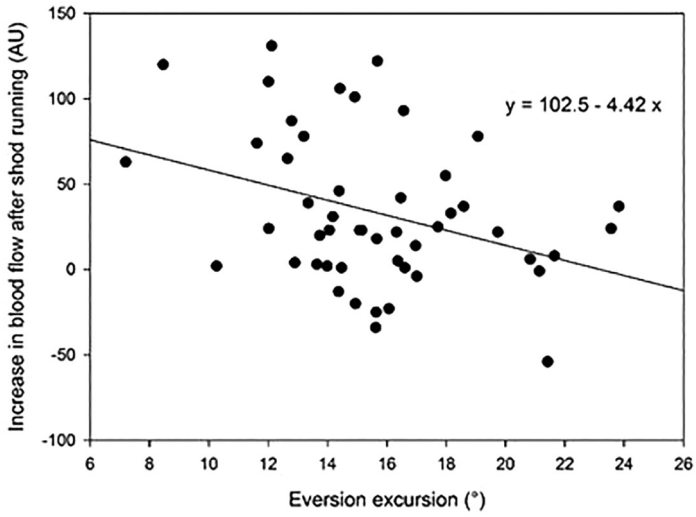


Fig. 4 Effect of eversion excursion on the increase in blood flow after shod running.

The dorsiflexion excursion did not significantly influence the increase in flow after shod running (F= 1.23, p= 0.273). Furthermore, the analysis of the foot strike classification showed that foot strike classification did not significantly affect the increase in flow after shod running (F= 0.04, p= 0.958). No significant effect on the increase in blood flow was found for the average or maximal velocities of both eversion and dorsiflexion excursion (F= 0.08, p= 0.774; F= 2.52, p= 0.119; F= 0.04, p= 0.838; F= 0.03, p= 0.870; respectively). Also, the interaction effect between all the kinematic variables and possible covariates was investigated and did not significantly influenced the increase in blood flow after running. In order to try to build a multivariate regression model, a manual forward selection procedure was used, but no other variables could be added next to the eversion excursion.

Discussion

The results of this study showed a significant effect of eversion excursion on the increase in blood flow during shod running. More specifically, the more eversion excursion was observed, the lower the increase in blood flow. Interestingly, this study also showed that the dorsiflexion excursion or the running foot strike pattern did not influence the increase in blood flow after running. Our results confirm one aspect of the whipping phenomenon hypothesis, as described by Clement et al[6], stating that foot pronation can cause partial constriction of the Achilles vascular network. These authors suggested that excessive foot pronation creates a whipping of torsional action upon the Achilles tendon as a result of substantial subtalar joint movement, since the foot rotates from an inverted position at heel strike to an everted position in midstance.

Pronation is defined as a triplanar motion involving a combination of rearfoot eversion, dorsiflexion and abduction of the foot.[30] Our study measured eversion of the foot. Eversion is defined as an inward rotation of the foot with respect to the longitudinal foot axis. The clinical axes of the foot are easy to determine in real life and allow simple biomechanical measurements that are an indicator of pronation and supination. Eversion and inversion are used in almost all biomechanical studies related to sport shoes. In most cases, where authors use the term "pronation", their statements are based on measurements of eversion.[24, 36] Also, this study measured eversion excursion, which is defined as the difference between the Achilles tendon angle at initial contact and the maximal eversion angle. This includes the subtalar joint movement from the inverted position at heel strike to the everted position in midstance, as described above in the whipping hypothesis.

As stated, previous research solely focused on the association between pronation and the development of the Achilles tendinopathy.[7, 27] As a result of the difficulties in measuring pronation during dynamic activities, this association between pronation and AT has not been frequently measured. Only two studies have investigated this relationship during dynamic activities. Ryan et al[27] investigated the differences in kinematic profiles of 21 healthy runners and 27 runners wit AT, using 3D motion capture system to analyze tri-plane kinematic data for the lower extremity. The results of this cross-sectional study indicated that during barefoot running, subjects with AT demonstrated greater rearfoot eversion during midstance than controls, with a trend towards greater overall rearfoot motion. Next, Donoghue et al[7] also used a cross-sectional study design with 11 AT patients and 11 healthy control and asked subjects to run barefoot and shod at a self-selected speed. The results of this study revealed a greater eversion in the AT group and also showed that running kinematics were exaggerated in shod condition compared with barefoot conditions.

Since our study stated that the eversion excursion significantly influences the Achilles tendon blood flow after running, and based on the results of these previous studies that subjects with AT showed greater eversion during running, we suggest considering anti-pronation measures to inhibit excessive pronation during running. Also, the prevention of increased inversion at initial contact might be useful, since an increased rearfoot inversion at heel strike will exhibit greater pronation from heel strike into midstance during gait. Previous research stated that pronation is at its maximum in the stance phase, but is already initiated during touchdown by the everting effect of ground reaction force that acts laterally from the subtalar joint axis.[14] We therefore suggest that anti-pronation measures, such as rigid or elastic anti-pronation taping techniques or orthoses or plantar intrinsic foot muscle exercises[10, 22], combining reduction of inversion at touch down and reduction of eversion around midstance, could be useful in managing and potentially even in preventing Achilles tendinopathy.

Several anti-pronation measures have been suggested. Anti-pronation taping techniques, such as the low-Dye and augmented Low-Dye, have consistently demonstrated reductions in vertical mobility of the midfoot and increased medial longitudinal arc height immediately following application. These anti-pronation taping techniques have traditionally been applied using a rigid sports tape, however in last decades there has been an increase in the use of elastic tapes, such as Kinesio tape™, Dynamic tape™, KT-tape ™, SpiderTech ™, and Cure tape ™.[10, 29, 35] Several studies in patients with Achilles tendinopathy showed an improvement of symptoms using insoles or orthoses.[8, 19, 23] The hypothesized mechanism is that these measures

reduce the bending stress within the Achilles tendon by correcting abnormal eversion of the calcaneus in the presence of excessive foot pronation,[17] or increase rearfoot movement variability.[8] However, recent discussions focus on optimization of muscular- regulated joint stability. In this sense, a combination of longitudinal arch support with rearfoot stabilization possibly leads to a modulation of the afferent input.[19] Furthermore, plantar intrinsic foot muscle training consisting of submaximal flexion of the metatarsophalangeal and proximal interphalangeal joints in order to raise medial longitudinal arch height, has also demonstrated reductions in vertical midfoot mobility in an asymptomatic population.[22] In addition, there is evidence that a whole limb approach to control midfoot mobility may be warranted.[10]

Interestingly, the significant effect of eversion on blood flow was only observed after shod running. A possible explanation is the duration of the running activity. To guarantee a valid study, this study was performed at Runners Service Lab, a specialized running Lab, making this study an experimental semi-field study. The standard protocol of Runners Service Lab was applied, consisting of barefoot running trials followed by shod running trials. Since the running activity was performed in non-randomized order, the influence of the running activity on blood flow was higher after shod running compared to after barefoot running. Therefore, significant results after shod running might be caused by an accumulated effect on blood flow because of the longer duration of the running activity. Another possible explanation is that pronation of the foot is increased in running shoes.[21] Willems et al[34] observed that during the shod running condition the eversion excursion increased by approximately 6° when wearing neutral shoes compared to barefoot running. Furthermore, Donoghue et al[7] stated that an increased eversion is often present in AT but may only become excessive when footwear is worn. These results dovetail nicely with our findings, since this study also found a greater eversion excursion during shod running, possibly explaining the significant effect of eversion on blood flow only after shod running (Table 2). Since running kinematics are exaggerated in shod condition, we suggest that it is clinically more relevant to investigate patients in shod condition during dynamic activities.

Another interesting finding in this study showed that the excursion in the sagittal plane or the running foot strike pattern did not significantly influenced blood flow after running. Although comparison to previous research is difficult, this finding is surprising since pronation is a triplanar motion involving a combination of rearfoot eversion, dorsiflexion and abduction of the foot.[30] Also, it is shown that foot strike pattern is related to both dorsiflexion and eversion range of motion, since both aspects are shown to be larger in natural forefoot strikers.[25] Therefore, it was reasonable to assume that the foot strike pattern during running would significantly

influence tendon blood flow after running. However, we can conclude that the results of this study demonstrate that the eversion excursion significantly influences tendon blood flow after shod running, whereas dorsiflexion excursion or foot strike pattern does not.

Several limitations of this study are noteworthy. The variability of marker placement and movement of skin markers over the underlying skeletal segments are well known issues. Furthermore, there are specific difficulties in the measurement of foot motion when wearing shoes.[2, 31] However, despite these limitations, this procedure remains frequently used for kinematic studies. Another limitation is that the blood flow measurements were performed directly after the running activity since real-time measurement of the blood flow is not possible. Finally, it is important to note that rearfoot eversion, although frequently used as a measurement for pronation, is only one aspect of pronation. Pronation is defined as a triplanar motion, and since our study obtained kinematic data in sagittal and frontal plane, further studies are required to examine the transverse plane motion and provide a more complete and more accurate analysis of pronation. Despite several limitations, this study is the first to report that the more eversion excursion during running, the lower the increase in blood flow after shod running. We therefore conclude that our findings support the hypothesis of the whipping phenomenon, stating that foot pronation creates a torsional action upon the Achilles tendon causing constriction of the Achilles vascular network. Further research should focus on the etiology of these findings in Achilles tendon injuries, by using a prospective study design. Also, in addition to the immediate post-running blood flow measurement, a follow-up measurement of the Achilles tendon blood flow for a long period after the running tasks could add a valuable contribution.

Perspectives

A widely cited hypothesis concerning the etiology of AT suggests that, in excessive pronation, the rotation of the tendon is accentuated and causes the Achilles tendon to undergo a "whipping" action which causes a decrease in blood flow.[6] The results of this study confirm that the more eversion excursion during running, the lower the increase in blood flow after shod running. We can conclude that our findings support the hypothesis of the whipping phenomenon. We therefore suggest that anti-pronation measures, to inhibit excessive pronation during running, could be useful in both preventing as managing Achilles tendinopathy. Furthermore, since the results were only seen after shod running, we suggest that it is clinically more relevant to investigate patients in shod condition during dynamic activities.

Acknowledgements: The authors gratefully acknowledge Jempi Wilssens and Philippe Vermaelen for the use of the research setting and the assistance in the kinematic data collection, and Tanneke Palmans for her assistance in analyses of the data.

References

1. Altman AR, Davis IS. A kinematic method for footstrike pattern detection in barefoot and shod runners. *Gait Posture.* 2012;35(2):298-300.
2. Arndt A, Wolf P, Liu A, et al. Intrinsic foot kinematics measured in vivo during the stance phase of slow running. *J Biomech.* 2007;40(12):2672-2678.
3. Azevedo LB, Lambert MI, Vaughan CL, O'Connor CM, Schwellnus MP. Biomechanical variables associated with Achilles tendinopathy in runners. *Br J Sports Med.* 2009;43(4):288-292.
4. Boesen MI, Koenig MJ, Torp-Pedersen S, Bliddal H, Langberg H. Tendinopathy and Doppler activity: the vascular response of the Achilles tendon to exercise. *Scand J Med Sci Sports.* 2006;16(6):463-469.
5. Breine B, Malcolm P, Van Caekenberghe I, Fiers P, Frederick EC, De Clercq D. Initial foot contact and related kinematics affect impact loading rate in running. *J Sports Sci.* 2017;35(15):1556-1564.
6. Clement DB, Taunton JE, Smart GW. Achilles tendinitis and peritendinitis: etiology and treatment. *Am J Sports Med.* 1984;12(3):179-184.
7. Donoghue OA, Harrison AJ, Laxton P, Jones RK. Lower limb kinematics of subjects with chronic achilles tendon injury during running. *Res Sports Med.* 2008;16(1):23-38.
8. Donoghue OA, Harrison AJ, Laxton P, Jones RK. Orthotic control of rear foot and lower limb motion during running in participants with chronic Achilles tendon injury. *Sports Biomech.* 2008;7(2):194-205.
9. Forst T, Hohberg C, Tarakci E, Forst S, Kann P, Pfutzner A. Reliability of lightguide spectrophotometry (O2C) for the investigation of skin tissue microvascular blood flow and tissue oxygen supply in diabetic and nondiabetic subjects. *J Diabetes Sci Technol.* 2008;2(6):1151-1156.
10. Franettovich M, Chapman A, Blanch P, Vicenzino B. A physiological and psychological basis for anti-pronation taping from a critical review of the literature. *Sports Med.* 2008;38(8):617-631.
11. Ghazanfari M VL, Banzer W, Rhodius U. Reproducibility of non-invasive blood flow measurements using laser Doppler spectroscopy. *Phys Med Rehab Kuror.* 2002(12):330-336.
12. Haglund-Akerlind Y, Eriksson E. Range of motion, muscle torque and training habits in runners with and without Achilles tendon problems. *Knee Surg Sports Traumatol Arthrosc.* 1993;1(3-4):195-199.
13. Hein T JP, Wagner-Fritz U, haupt G, Grau S. Prospective analysis of intrinsic and extrinsic risk factors on the development of Achilles tendon pain in runners *Scand J Med Sci Sports.* 2014(24):201-2012.
14. Kalin VX, Denoth J, Stacoff A, Stussi E. [Running injuries and running shoe construction: demonstration of possible correlations]. *Sportverletz Sportschaden.* 1988;2(2):80-85.
15. Langberg H, Olesen J, Skovgaard D, Kjaer M. Age related blood flow around the Achilles tendon during exercise in humans. *Eur J Appl Physiol.* 2001;84(3):246-248.
16. Lopes AD, Hespanhol Junior LC, Yeung SS, Costa LO. What are the main running-related musculoskeletal injuries? A Systematic Review. *Sports Med.* 2012;42(10):891-905.
17. Maffulli N, Kader D. Tendinopathy of tendo achillis. *J Bone Joint Surg Br.* 2002;84(1):1-8.
18. Mahieu NN, Witvrouw E, Stevens V, Van Tiggelen D, Roget P. Intrinsic risk factors for the development of achilles tendon overuse injury: a prospective study. *Am J Sports Med.* 2006;34(2):226-235.
19. Mayer F, Hirschmuller A, Muller S, Schuberth M, Baur H. Effects of short-term treatment strategies over 4 weeks in Achilles tendinopathy. *Br J Sports Med.* 2007;41(7):e6.
20. McCrory JL, Martin DF, Lowery RB, et al. Etiologic factors associated with Achilles tendinitis in runners. *Med Sci Sports Exerc.* 1999;31(10):1374-1381.
21. Michel FI, Kalin X, Metzger A, et al. [The functional sport shoe parameter "torsion" within running shoe research--a literature review]. *Sportverletz Sportschaden.* 2009;23(4):197-205.
22. Mulligan EP, Cook PG. Effect of plantar intrinsic muscle training on medial longitudinal arch morphology and dynamic function. *Man Ther.* 2013;18(5):425-430.
23. Munteanu SE, Scott LA, Bonanno DR, et al. Effectiveness of customised foot orthoses for Achilles tendinopathy: a randomised controlled trial. *Br J Sports Med.* 2015;49(15):989-994.
24. Nigg BM. *Biomechanics of sports shoes.* Canada: Topline Printing Inc; 2010.
25. Nunns M, House C, Fallowfield J, Allsopp A, Dixon S. Biomechanical characteristics of barefoot footstrike modalities. *J Biomech.* 2013;46(15):2603-2610.
26. R M. Achilles tendonitis. *Foot Ankle Clin.* 1997(2):439-456.

27. Ryan M, Grau S, Krauss I, Maiwald C, Taunton J, Horstmann T. Kinematic analysis of runners with achilles mid-portion tendinopathy. *Foot Ankle Int.* 2009;30(12):1190-1195.
28. Smart GW, Taunton JE, Clement DB. Achilles tendon disorders in runners--a review. *Med Sci Sports Exerc.* 1980;12(4):231-243.
29. Smith M, Brooker S, Vicenzino B, McPoil T. Use of anti-pronation taping to assess suitability of orthotic prescription: case report. *Aust J Physiother.* 2004;50(2):111-113.
30. Stacoff A, Reinschmidt C, Nigg BM, et al. Effects of foot orthoses on skeletal motion during running. *Clin Biomech (Bristol, Avon).* 2000;15(1):54-64.
31. Stacoff A, Reinschmidt C, Stussi E. The movement of the heel within a running shoe. *Med Sci Sports Exerc.* 1992;24(6):695-701.
32. Theobald P, Benjamin M, Nokes L, Pugh N. Review of the vascularisation of the human Achilles tendon. *Injury.* 2005;36(11):1267-1272.
33. Walter B, Bauer R, Krug A, Derfuss T, Traichel F, Sommer N. Simultaneous measurement of local cortical blood flow and tissue oxygen saturation by Near infra-red Laser Doppler flowmetry and remission spectroscopy in the pig brain. *Acta Neurochir Suppl.* 2002;81:197-199.
34. Willems TM, Witvrouw E, De Cock A, De Clercq D. Gait-related risk factors for exercise-related lower-leg pain during shod running. *Med Sci Sports Exerc.* 2007;39(2):330-339.
35. Williams S, Whatman C, Hume PA, Sheerin K. Kinesio taping in treatment and prevention of sports injuries: a meta-analysis of the evidence for its effectiveness. *Sports Med.* 2012;42(2):153-164.
36. Wyndow N, Cowan SM, Wrigley TV, Crossley KM. Neuromotor control of the lower limb in Achilles tendinopathy: implications for foot orthotic therapy. *Sports Med.* 2010;40(9):715-727.

CHAPTER III

The role of the vascular and structural response to activity in the development of Achilles tendinopathy

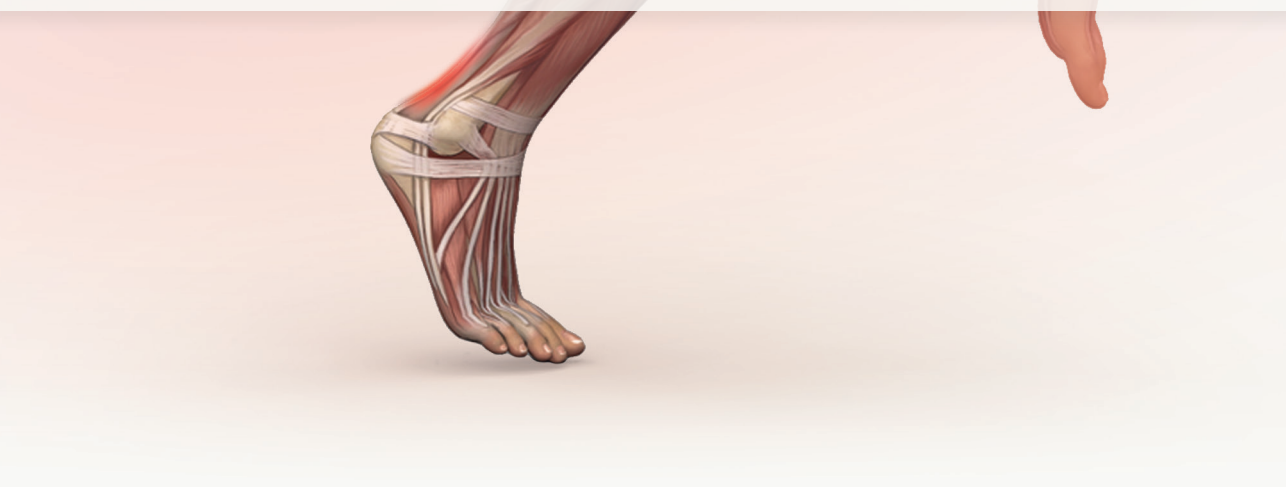
A prospective study

E. Wezenbeek[1], TM. Willems[1], N. Mahieu[1], M. De Muynck[2], L. Vanden Bossche[2], A. Steyaert[2], D. De Clercq[3], E. Witvrouw[1]

[1] Department of Rehabilitation Sciences and Physiotherapy, Ghent University, Ghent, Belgium,
[2] Department of Physical and Rehabilitation Medicine, Ghent University Hospital, Ghent, Belgium,
[3] Department of Movement and Sport Sciences, Ghent University, Ghent, Belgium

Accepted for publication in American Journal of Sports Medicine, 2017
Original article (SCI 5.673, Q1) Ranking: 1/75 (Orthopedics)

Full reference:
The Role of the Vascular and Structural Response to Activity in the Development of Achilles Tendinopathy: A Prospective Study. Wezenbeek E, Willems T, Mahieu N, De Muynck M, Vanden Bossche L, Steyaert A, De Clercq D, Witvrouw E. Am J Sports Med. 2018 Mar;46(4):947-954.



Abstract

Several risk factors have been suggested in the development of Achilles tendinopathy, but large scale prospective studies are limited. Therefore, the aim of this cohort study was to investigate the role of the vascular response to activity of the Achilles tendon, tendon thickness, ultrasound tissue characterisation (UTC) of tendon structure, and foot posture as possible risk factors in the development of Achilles tendinopathy. The study began with 351 first-year students at Ghent University. After 51 students were excluded, 300 were tested in the academic years 2013-2014 and 2014-2015 and were followed prospectively for 2 consecutive years by use of a multilevel registration method. Of those, 250 students were included in the statistical analysis. At baseline, foot posture index and UTC were investigated bilaterally. Blood flow and tendon thickness were measured before and after a running activity. Cox regression analyses were performed to identify significant contributors to the development of Achilles tendinopathy. During the 2-year follow-up, 27 of the included 250 participants developed Achilles tendinopathy (11%). Significant predictive effects were found for female sex and blood flow response after running ($p=0.022$ and $p=0.019$, respectively). The risk of developing Achilles tendinopathy increased if the blood flow increase after running was reduced, regardless of sex, foot pronation and timing of flow measurements. The model had a predictive accuracy of 81.5% regarding the development of Achilles tendinopathy, with a specificity of 85.0% and a sensitivity of 50.0%. This prospective study identified both female sex and the diminished blood flow response after running as significant risk factors for the development of Achilles tendinopathy. UTC of tendon structure, Achilles tendon thickness and foot posture did not significantly contribute to the prediction of Achilles tendinopathy. A general evaluation of tendon structure by UTC, measurement of tendon thickness or determination of the foot posture index will not allow clinicians to identify patients at risk for developing Achilles tendinopathy. Furthermore, it may be possible to improve blood flow after activity using non-invasive techniques designed (such as prostaglandins, heat, massage, vibration techniques et cetera). These techniques may be useful in the prevention and management of Achilles tendinopathy, but further research is needed.

Key Terms: Prospective study, risk factors, Achilles tendinopathy, blood flow, thickness, foot posture, ultrasound tissue characterisation (UTC), running

Introduction

Although the Achilles tendon is the largest and strongest tendon in the human body, injuries are common.[41] The most common Achilles tendon injury is tendinopathy.[2] Despite the high prevalence and the often deleterious consequences of Achilles tendinopathy (AT), many aspects of its aetiology remain unknown.

Several risk factors for AT have been suggested, but large scale prospective studies are lacking.[7, 13, 29, 45] Although both structural and vascular factors have been suggested as risk factors for the development of AT, it remains unclear whether these structural or vascular parameters predict future symptoms. Previous studies already reported the need for longitudinal studies to investigate the physiological or pathological effect of vascularization after activity and its predictive value in the development of AT.[6, 33] Furthermore, the identification of Achilles tendon abnormalities, visualized by ultrasound imaging (US), showed conflicting results as risk factor for the development of AT, since these structural abnormalities could be caused by load and are also present in large percentage of asymptomatic sporting populations.[8, 16, 21, 28] Also, a relatively new technique was developed to optimize the visualization of tendon structure.[48] This novel technique, ultrasound tissue characterisation (UTC), has been demonstrated to be suitable to quantify subtle changes in tendon structure.[10-12, 44, 48, 51] Despite increasing publications on the use of UTC, it is striking that, to date, no prospective studies have been published.

The lack of clarity on specific risk factors has led to considerable uncertainty in the management and prevention of AT. Therefore, the aim of this prospective study was to investigate the role of the Achilles tendon thickness, vascular response to activity, foot posture and UTC tendon structure as possible risk factors in the development of Achilles tendinopathy.

Materials and methods

Study design

In this prospective cohort study, at the start of each of the two academic years, blood flow, Achilles tendon thickness, ultrasound tissue characterisation, and foot posture index© (FPI) evaluations of both Achilles tendons were performed in randomized order in all subjects. Then, participants were instructed to perform a physical activity, and thereafter the blood flow measurements and ultrasound examination were repeated. Participants were instructed not to engage in any sports activities 48 h prior to testing to ensure a valid resting state measure. The subjects also

completed a questionnaire concerning demographic and anthropometric data, sport activities, and injury history. After these measurements, injury registration was performed during a follow-up period of 2 consecutive years, or until the end of the academic year of 2015-2016. For 29 weeks per academic year the participants followed the same sports program under similar environmental conditions as part of their education. The average weekly sports participation (sum of basic sports education, practice hours, and sports participation outside the official educational program) was registered and this total amount of sports participation was individually used as time at risk for every subject. The time at risk was registered from the start of the study until the injury, or the end of the study for students who did not develop an injury. In case of dropout, the time until last personal contact was taken into account for the individual time at risk. This study was approved by the Ethics Committee of the Ghent University Hospital (number of approval: EC/2013/616).

Participants

In this study, 351 freshmen students at Ghent University participated. Participants were excluded from this study if they had a history of AT, surgery of the lower extremity or severe trauma in the lower extremity (LE) within the previous year. This resulted in 300 included participants (141 male and 159 female), with a mean age of 18.0$\pm$0.8 years (Table 1). All students were evaluated in the academic years 2013-2014 and 2014-2015. Of those, 250 were taken into account for statistical analysis since 50 students developed other LE injuries (no AT) and were therefore excluded from the study. Of the 250 participants, 27 developed AT and 223 subjects did not sustain any injury of the LE and were used as control subjects (Figure 1).

Blood flow measurement

Blood flow data at the midportion of the Achilles tendon were collected by use of the oxygen-to-see® (O2C) device (LEA Medizintechnik Giessen, Germany). This non-invasive device is capable of measuring the perfusion and oxygenation of the subcutaneous tissue using an optical flat fiber probe. Measurements were performed as described in Wezenbeek et al.[52] at a depth of 8mm. The head of the probe was placed flat on the tissue at the measurement point, without pressure or movement. For standardization purposes, measurements were performed in a darkened room with constant temperature. The stability of the blood flow measurements has been investigated and the measurement has been shown to be reliable and valid.[15, 52] A two-way mixed single measures intra-class correlation (ICC) for absolute agreement as a measure for intra observer reliability was 0.75 and minimal detectable change was calculated as 9.2 arbitrary units (AU).

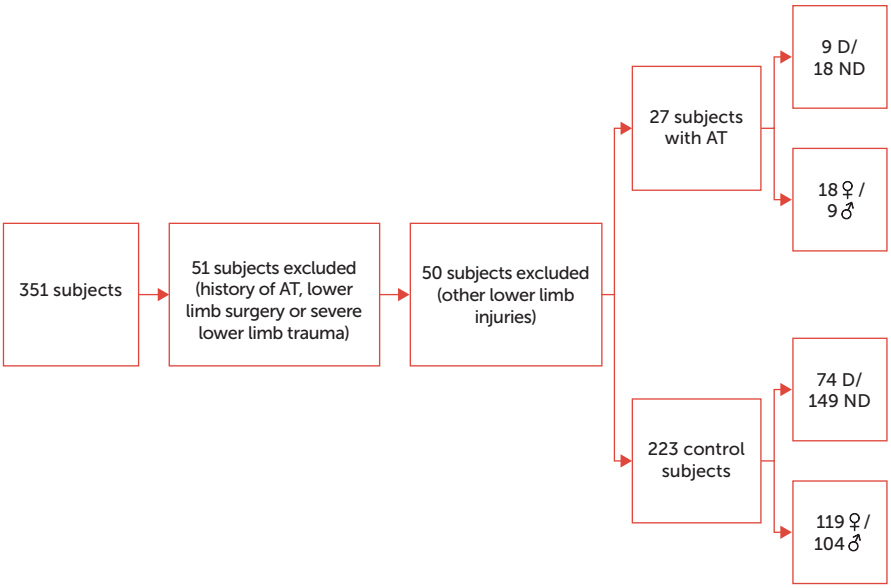


Fig. 1 Flowchart to identify the injured group with Achilles tendinopathy (AT) and the randomly selected dominance-matched control group.

Achilles tendon thickness

Bilateral gray-scale ultrasonography examinations of the thickness of the Achilles tendons were performed using a high resolution linear array 11.4 MHz ultrasound transducer (Siemens Acuson X150, Siemens NV, Erlangen, Germany and Logic Scan 128, Telemed, Vilnius, Lithuania). Participants were placed in prone position with both feet hanging free outside the examination table. The thickness was assessed in the transverse plane and the true tendon thickness was measured at distances of 2 and 5 cm proximal to the calcaneal border. The reliability of the thickness measurements was investigated in ten Achilles tendons of age-matched subjects on two different measurement days in the same week. Intra-class correlation (ICC) was 0.78 and 0.83 at 2 and 5 cm, respectively, and minimal detectable changes were calculated as 0.4 mm for both 2 and 5 cm.

Ultrasound Tissue Characterisation (UTC)

Tissue characterisation was performed in all participants. The protocol for this measurement was described in detail by Wezenbeek et al.[51] For analysis, the midportional volume 2-6 cm proximal of the calcaneus, starting from the proximal border of the

calcaneus, was used. Images were analyzed by selecting a region of interest (ROI), defined by the outline of the Achilles tendon in the transverse plane. Nine ROIs were placed in the longitudinal plane at regular intervals of 5 mm. Contours were then interpolated between these contiguous ROIs creating a tendon volume of the mid-portion (2-6 cm), in which the proportions of echo-types were quantified. Four valid echo-types can be discriminated: echo-type I represents intact, continuous and aligned fibers and fasciculi, echo-type II represents less continuous and/or more wavy fibers and fasciculi, echo-type III represents a mainly fibrillar matrix and echo-type IV represents complete disintegration, with tendon tissue replaced by an amorphous matrix and fluid.[48] The window size used for interpolation was 17, i.e. tendon structure was quantified with dedicated UTC algorithms that assessed the echopattern by means of relative intensity and distribution of grey levels of corresponding pixels over 17 images (3.2 mm). Test-retest reliability was investigated and ICCs for intra observer reliability were between 0.96 and 0.98 and minimal detectable changes were calculated as 7.0%, 6.5%, 0.8% and 0.2% for the four echo-types, respectively.[51]

Foot Posture Index©

The foot posture was quantified using the Foot Posture Index© (FPI-6), a six item foot posture assessment tool. The assessment was conducted as described in Redmond et al.[42] High positive aggregate values (values between +6 and +12) indicate a pronated posture, more neutral (0 to +5) and negative values (-1 to -12) indicate respectively more neutral or supinated foot posture.[42] The assessment tool has been shown to be reliable and valid.[30, 42]

Physical activity

Participants were instructed to run 1.92 km at an average running pace of 3.2m/s. Participants ran shod on a Finnish track, wearing self-selected running shoes.

Injury registration and diagnostic criteria

Since the injury registration method and diagnostic criteria are very important in injury recording,[3] a multilevel registration method and accurate diagnostic criteria were used. A primary online registration method was used to identify students with Achilles tendon pain or dysfunction. For this online method, the participants received a weekly reminder by email to register their injuries in an online questionnaire. When subjects presented with a Achilles tendon complaint, further diagnosis of the AT was performed by an experienced MD. More specifically, one or more of the following criteria were used for diagnosing AT: (1) an atraumatic occurrence of non-insertional Achilles tendon pain, exacerbated by running, (2) the presence of swelling at the midportion of the Achilles tendon, (3) non-insertional pain, ache or soreness of the Achilles tendon with possible functional limitation

during physical active participation.[25, 47] Every three months participant interviews were conducted to check the compliance of the injury registration and if needed to confirm for the occurrence of AT.

Statistical analysis

The injured leg of the participants who developed AT was used in the statistical analysis. If a participant developed bilateral symptoms, only the more 'painful' tendon was included, based on the visual analogue score. The injured legs were matched with legs of the control group such that the percentage of dominant legs in the control group matched with the percentage of dominant legs in the injured group. Therefore, one leg per participant of the control group was randomly selected until the ratio of non-dominant/dominant legs of the control group was equal to the injured group (Figure 1).

Statistical analysis was done with the SPSS V.23 Statistical Software package (IBM Corp., New York, USA). Firstly, univariate cox regression analyses were performed allowing reduction of the number of variables since variables were only included for further analysis if $p<0.2$. Secondly, stepwise multivariate cox regression analyses were performed to identify significant contributors to the development of AT while correlations were checked between covariates to exclude multicollinearity. Variables with a $p<0.05$ in the Cox regression analysis, were seen as significant predictors for AT.

Results

During the follow-up period, 27 of the 250 included participants developed AT (11%). A total of 223 of the 250 participants did not sustain any injuries of the lower leg and served as the control group (89%). The AT injury rate was 0.3 events per 1000 hours sports participation. Subject demographics, tendon characteristics and results of the univariate cox regression can be found in Table 1.

Cox regression

In the model building, correlations were seen between height, weight, BMI, tendon thickness, and sex and also between blood flow after activity and the increase in blood flow after activity. After stepwise multivariate cox proportional hazards model building for the development of AT, the final model included the variables sex, increase in blood flow after running, timing of blood flow measurements and foot posture. The timing of blood flow measurements and foot posture were included as confounders in the analyses since previous studies showed that the Achilles

Table 1 Subject demographics and tendon characteristics for the uninjured and AT group.

	Control Group (n=223)[b]	AT Group (n=27)[b]	P-value
Age, y	18.03 ± 0.87	17.85 ± 0.53	0.374
Height, m	1.75 ± 0.08	1.72 ± 0.08	0.028
Weight, kg	64.43 ± 9.05	61.03 ± 7.01	0.015
Body Mass Index, kg/m^2	21.08 ± 2.04	20.52 ± 1.68	0.166
Rating of perceived exertion	5.6 ± 1.8	5.2 ± 1.4	0.465
Sport participation, hours/week	6.85 ± 4.90	8.64 ± 5.23	0.256
Midportion echo type I, % [6]	55.82 ± 15.17	55.48 ± 14.71	0.394
Midportion echo type II, % [6]	40.47 ± 12.21	40.49 ± 11.93	0.297
Midportion echo type III, % [6]	3.19 ± 5.85	3.47 ± 3.92	0.865
Midportion echo type IV, % [6]	0.53 ± 0.92	0.56 ± 0.67	0.655
Flow midportion pre, arbitrary units [3]	48.98 ± 19.33	47.87 ± 19.58	0.351
Flow midportion post, arbitrary units [3]	77.57 ± 34.46	66.71 ± 25.89	0.065
Flow midportion increase, arbitrary units [3]	28.71 ± 27.09	19.05 ± 23.19	0.146
Thickness, pre, 2 cm to calcaneus (mm) [1]	4.44 ± 0.61	4.21 ± 0.67	0.026
Thickness, pre, 5 cm to calcaneus (mm) [2]	3.61 ± 0.73	3.48 ± 0.68	0.548
Thickness, post, 2cm to calcaneus (mm) [1]	4.34 ± 0.62	4.12 ± 0.62	0.056
Thickness, post, 5 cm to calcaneus (mm) [2]	3.61 ± 0.78	3.60 ± 0.79	0.981
Sex, n (%)			0.013
Males	104/223 (47)	9/27 (33)	
Females	119/223 (53)	18/27 (67)	
Dominance, n (%)			0.805
Dominant leg	74/223 (33)	9/27 (33)	
Non – dominant leg	149/223 (67)	18/27 (67)	
Foot posture [3], n (%)			0.594
Pronated foot	41/221 (19)	4/26 (15)	
Neutral or supinated foot	180/221 (81)	22/26 (85)	

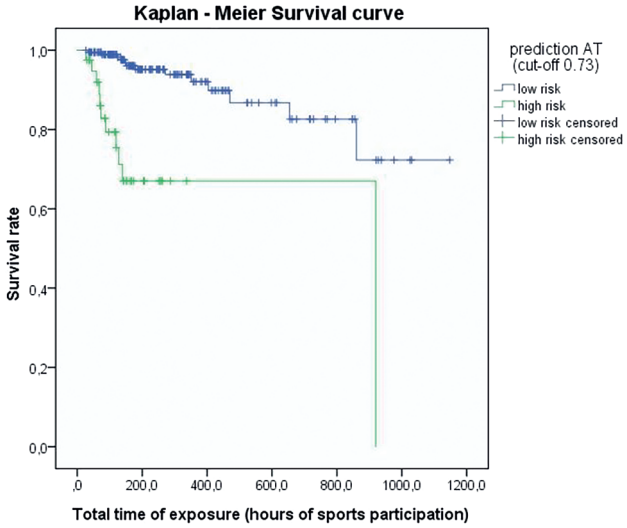
[a] Brackets indicate numbers of missing values. Pre and post refer to before and after the running activity.
[b] Data expressed as mean ± SD or n (%).
[c] P-values were obtained by means of univariate cox regression analyses

tendon blood flow after activity showed a fast increase followed by a quick recovery to baseline,[43] and since recent studies confirmed the relationship between foot pronation and Achilles tendon blood flow.[22, 52] The results of the multivariate Cox regression analysis and the strength of the predictive values are presented in Table 2.

Table 2 Risk model for the prediction of AT versus no injury obtained by multivariate Cox regression.

Indicator	B	SE	P-value	Hazard ratio	95% Confidence Interval
Increase in blood flow	-0.026	0.011	**0.019***	0.974	0.953-0.996
Pronation	-0.285	0.552	0.605	0.752	0.255-2.217
Instantly measured	0.552	0.594	0.353	1.736	0.542-5.562
Female sex	1.038	0.454	**0.022***	2.822	1.160-6.866

* p-value < 0.05



Number of events / Number at risk						
TOE (h)	0-200	200-400	400-600	600-800	800-1000	1000-1200
Low risk	6/194	2/95	2/43	1/23	1/11	0/3
High risk	12/43	0/11	0/1	0/1	1/1	/

Fig. 2 Kaplan-Meier Survival curve.

The multivariate Cox regression analysis showed a significant predictive effect of female sex and increase of blood flow after running. The hazard of developing AT increased by 3% if the blood flow increase after running is lowered with 1 arbitrary unit (AU), regardless of sex, foot pronation and timing of flow measurements. Based on the current model, prognostic scores were calculated and the cut-off value was determined at 0.73 to indicate a group with high prognostic score and a group with low prognostic score. The model had a predictive accuracy of 81.5% regarding the development of Achilles tendinopathy, with a specificity of 85.0% and a sensitivity of 50.0%. The Kaplan-Meier survival curve of the high and low risk group is displayed in Figure 2.

Discussion

The results of this study identified both female sex and the blood flow response after running as risk factors for the development of AT. Interestingly, in this study no association was found between AT and Achilles tendon thickness, UTC, or pronated foot posture.

Vascular parameters and injury risk

Physical activity generates an increased metabolic demand. In order to fulfill this increased metabolic demand a necessary cardiovascular redistribution of blood flow to the working soft tissues is induced.[14] Consequently, an increase in Achilles tendon blood flow as normal physiological response to activity has been observed.[37]

This study is the first to investigate this blood flow increase after running as a risk factor for the development of AT. The results of this study showed that the lower the increase in blood flow after running, the higher the hazard for developing AT. The difference in increase in blood flow between the AT and control group was greater than the minimal detectable change. Our results are in line with previous research showing that a decrease of the tendon blood supply results in hypoxia, which has been demonstrated to regulate inflammatory and apoptotic factors in tendon cells and to promote a "switch" in collagen matrix synthesis.[26] This resultant altering of material properties leads to weakening of the normal tendon structure.[39] In addition, it is suggested that in a hypovascular structure the extent of necessary matrix remodeling is restricted.[40] Also, it has been suggested that an insufficient blood supply could indicate anaerobic conditions in the tendon. This is supported by previous studies that found significantly higher lactate levels in pathological Achilles tendons compared with normal tendons.[1] Furthermore, Pufe et al.[39] stated that the recovery process takes longer in hypovascular tissue and there is a higher

chance that a damaging force is reapplied before this recovery occurs. Therefore, it is not very surprising that a lower increase in blood flow after running is associated with AT. Indeed, it is plausible that a lower increase in blood flow after running creates insufficient or delayed regeneration of the tendon due to insufficient recovery in between training loads, explaining the higher hazard for developing AT. Although previous research stated that there is no significant difference in blood flow values before and after activity between this population and an older population[24] and it can therefore be assumed that this risk factor is not age-dependent, further research is indicated to establish this.

Since this study was the first study to identify an association between a lower increase in blood flow after running and the development of AT, it seems logical that the increase in blood flow after activity should be stimulated. First, it is possible that the participants with a lower increase in blood flow after running are in need of a longer warming-up to improve the vascular response to activity. It is stated that warming-up before a sport event enhances performance and decreases injury risk since an induced increase in body temperature leads to vasodilation of the blood vessels in the working soft tissues, subsequently leading to an increase in blood flow.[20] Next, prostaglandins are indicated to elevate blood flow during increased metabolic demands such as exercise.[5] Also, compression stockings, heat, massage and vibration techniques are suggested to increase the blood flow and therefore might facilitate the recovery process after activity.[17, 23, 38, 49, 50] However, further research is needed to investigate the vascular response of these latter techniques in the Achilles tendon since current literature is limited to changes in blood flow in the skin surface. Also, since the results of this study identified that the hazard for developing AT is higher when the increase in blood flow after running is lowered, influencing factors of this vascular response, such as smoking, BMI, diabetes, tendon structure, high cholesterol, genetics, nutrition and age should be further investigated. Prior research showed that more eversion excursion during running showed a significant decrease in Achilles tendon blood flow after running and measures that decrease pronation during running are suggested to be useful in both preventing and managing Achilles tendinopathy.[52] Although further research is needed to ensure that reducing eversion excursion increases blood flow.

Sex and injury risk

This was the first study to investigate sex as a risk factor for the development of AT. Although sex is often mentioned as a risk factor for Achilles tendinopathy (particularly men are suggested to be at higher risk), it is remarkable that this has never been studied prospectively. The results of this study found an association between female sex and an increased hazard for the development of AT. It is possible that sex

differences in AT incidence may simply be the result of sex-related differences in reporting injuries or seeking care.[34] Also, it could be that women in our study were less resistant to withstand the increased load of the sports program compared to their male counterparts, since no difference in physical activity was made for sex in their educational program. Magnan et al.[26] also suggested that the higher incidence of AT reported in males may not reflect higher susceptibility of male sex to AT, since it must be considered that differences in physical activity make it difficult to evaluate sex as an independent etiological factor. Therefore, it is plausible that, in a young adult population, women are at higher risk to develop AT when performing a program with similar load. This finding should be considered when imposing a similar load program on both males and females, such as physical activity in the army or in educational programs.

Structural parameters and injury risk

Interestingly, this study did not identify structural parameters, such as the Achilles tendon thickness and tendon structure as defined by UTC, as predictive parameters for the development of AT. Despite increasing publications on the use of UTC, it is striking that, to date, this present study is the first to prospectively investigate the role of tendon structure, defined by UTC, in the development of AT. In this study the amount of echo-type II could not be identified as a risk factor for the development of AT in active, healthy young adults. This is not surprising since echo-type II tendon bundles are not pathological and can be considered as a normal physiological finding in an active, adolescent population.[51] In contrast, prior research identified the presence of echo types III and IV as indicating inferior tendon quality and potentially a pathological tendon.[48] In our study, the presence of echo-types III and IV was marginal (3.88 ± 6.63 and 0.77 ± 1.38 percentages, respectively). As a consequence, the results of this study do not allow a conclusion concerning the increased risk of AT based on the presence of echo-types III and IV. It is possible that the evaluation of the entire midportional volume is not suitable to quantify subtle differences in tendon structure that may precede the development of symptoms. Furthermore, previous research stated that UTC tendon structure differs between sexes, and different locations in the tendon should be taken into account.[51] Next, our results were not able to identify the Achilles tendon thickness as a possible risk factor for the development of AT. The reported tendon thickness in this study was in accordance to previous studies[8, 18], however, comparison to previous literature is difficult since the US materials and measurement techniques vary between studies. Several longitudinal studies have investigated whether tendon thickness in asymptomatic individuals is predictive for future symptomatic tendinopathy, with conflicting results. The results of this study are in agreement with Comin et al.[8] who investigated tendon thickness in 79 professional ballet

dancers and found no correlation between thickness and the development of symptoms. Also, Giombini et al.[18] and Hirschmuller et al.[19] examined Achilles tendons in 37 elite fencers and 634 runners, respectively, and also found that Achilles tendon thickness could not predict the development of symptoms. In contrast, two studies found that soccer/football players with thicker Achilles tendons were more at risk to develop symptoms, namely Ooi et al.[36] who tested 42 elite Australian football players with a mean age of 24 (18-34) years, and Jhingan et al.[21] who tested 18 elite soccer players with a mean age of 23.5 (22-27.5) years. The conflicting result with these two studies might possibly be explained by our study population. It is possible that our results did not identify tendon thickness as a risk factor in this active, young adult population since participants with history of Achilles tendon complaints were excluded in this study (in contrast to the population tested by Jhingan et al.[21]). This indicates that AT diagnosed in this study is probably initial tendon pathology and not advanced tendon pathology, while the increased tendon thickness is more frequently associated with tendon degeneration, and this stage is primarily seen in the older persons or in persons with previous complaints.[9, 27] Secondly, in this study true tendon thickness at fixed distances from calcaneal border was measured. This is in contrast with the study of Ooi et al.[36] who identified the maximum tendon thickness as a risk factor for the development of AT. Since we did not register the maximum tendon thickness, no conclusion regarding this possible risk factor could be drawn in our study.

Foot posture and injury risk

In this study, a pronated foot defined by the FPI© was not associated with an increased hazard for the development of AT. Previous studies identified the pronated foot posture as described by the Foot Posture Index© as a risk factor for various lower limb injuries, such as medial tibial stress syndrome, patellofemoral pain syndrome and ankle sprains but no relationship was identified between foot posture index© and AT.[32, 35] However, it has been suggested that excessive foot pronation creates a whipping or torsional action upon the Achilles tendon, which causes vascular blanching of the midportion of the Achilles tendon, as the foot rotates rapidly from an inverted position at heel strike to an excessively everted position in midstance.[7] This whipping hypothesis is supported by some retrospective studies, demonstrating greater rearfoot eversion and greater rearfoot eversion range of motion during running than controls.[13, 29, 45] Recent studies confirmed this relationship between foot pronation and Achilles tendon blood flow.[22, 52] Indeed, Wezenbeek et al.[52] showed that the more eversion excursion was observed, the lower the increase in blood flow after running.[52] Therefore, it is surprising that foot pronation, defined by the FPI©, could not be associated with an increased risk for the development of AT. A possible explanation is that the FPI©, assessed in static

position, was not sensitive enough as a predictor of eversion ROM during a dynamic running activity. Furthermore, although the FPI© has been shown to be a reliable and valid assessment tool by several authors[30, 42], some authors state that caution is needed when interpreting the FPI© results[46], more specifically when participants are categorized into a certain foot type based on their FPI© score since this may limit the sensitivity[4]. Also, the foot posture index© in this study is evaluated in barefoot condition, whereas pronation of the foot is increased in running shoes.[31]

Methodological considerations

This is the first large-scale prospective study to investigate the role of the Achilles tendon thickness and vascular response to activity, foot posture, and UTC tendon structure as possible risk factors in the development of Achilles tendinopathy. This was studied in an active, young adult population to minimalize the age-related degeneration of the tendon, making it possible to investigate these parameters as independent etiological factors.

Several limitations of this study are noteworthy. Since an association between Achilles tendon blood flow and kinematic variables has been shown, measuring limb kinematics would have given this study an added value. However, the testing protocol of this large-scale prospective study was already time-consuming, therefore the foot posture index© was implemented. Furthermore, the implementation of a validated overuse questionnaire such as the Oslo Sports Trauma Research Centre (OSTRC) would have been an added value to this study, as well as the weekly workload registration to define the acute: chronic ratio. Another limitation is that the blood flow measurements were performed directly after the running activity since real-time measurement of the blood flow is not possible. Also, blood flow measurements and Achilles thickness measurements were performed in randomized order after running. Since this increase in blood flow after activity is followed by a quick recovery to baseline, the differences in the timing of the blood flow measurement after activity is a limitation. We have however added timing of blood flow measurement as a confounder in our statistical analyses and are convinced that thereby we have overcome this limitation. Next, we are aware of the limited number of events to study several variables since the rule of thumb indicates that a cox model should use a minimum of 10 outcome events per predictor variable. Therefore, after our univariate analyses, we included a limited amount of predictors to not overfit our cox model. It should also be mentioned that the general UTC evaluation, whereby the midportional volume 2-6 cm proximal of the calcaneus was assessed, was possibly not suitable to detect small structural changes in the tendon. Therefore, the assessment of possibly present structural changes, e.g. hypoechoic regions, rather than assessing the entire midportion, could be more

suited as a predictor of further pathology. We also acknowledge the homogeneity of our study population of active young adults, which limits the generalization of these findings to other age groups. Nevertheless, this population was targeted to exclude age-related degeneration and represents normal, healthy tendons.

Conclusion

This study was the first study to prospectively identify both female sex and the blood flow response after running as significant predictors for the development of AT. Achilles tendon thickness, foot posture, and a general evaluation of the tendon structure, defined by UTC, were not significantly associated with the risk of developing AT. Possibly, a general evaluation of UTC tendon structure is not suitable to quantify subtle differences in tendon structure that may precede the development of symptoms in a healthy population of young adults.

References

1. Alfredson H, Lorentzon R. Intratendinous glutamate levels and eccentric training in chronic Achilles tendinosis: a prospective study using microdialysis technique. *Knee Surg Sports Traumatol Arthrosc.* 2003;11(3):196-199.
2. Asplund CA, Best TM. Achilles tendon disorders. *BMJ.* 2013;346:f1262.
3. Bahr R. No injuries, but plenty of pain? On the methodology for recording overuse symptoms in sports. *Br J Sports Med.* 2009;43(13):966-972.
4. Barton CJ, Levinger P, Crossley KM, Webster KE, Menz HB. Relationships between the Foot Posture Index and foot kinematics during gait in individuals with and without patellofemoral pain syndrome. *J Foot Ankle Res.* 2011;4:10.
5. Boushel R, Langberg H, Risum N, Kjaer M. Regulation of blood flow by prostaglandins. *Curr Vasc Pharmacol.* 2004;2(2):191-197.
6. Cassel M, Carlsohn A, Frohlich K, John M, Riegels N, Mayer F. Tendon Adaptation to Sport-specific Loading in Adolescent Athletes. *Int J Sports Med.* 2016;37(2):159-164.
7. Clement DB, Taunton JE, Smart GW. Achilles tendinitis and peritendinitis: etiology and treatment. *Am J Sports Med.* 1984;12(3):179-184.
8. Comin J, Cook JL, Malliaras P, et al. The prevalence and clinical significance of sonographic tendon abnormalities in asymptomatic ballet dancers: a 24-month longitudinal study. *Br J Sports Med.* 2013;47(2):89-92.
9. Cook JL, Purdam CR. Is tendon pathology a continuum? A pathology model to explain the clinical presentation of load-induced tendinopathy. *Br J Sports Med.* 2009;43(6):409-416.
10. De Jonge S, Warnaars JL, De Vos RJ, et al. Relationship between neovascularization and clinical severity in Achilles tendinopathy in 556 paired measurements. *Scand J Med Sci Sports.* 2014;24(5):773-778.
11. Docking SI, Cook J. Pathological tendons maintain sufficient aligned fibrillar structure on ultrasound tissue characterization (UTC). *Scand J Med Sci Sports.* 2016;26(6):675-683.
12. Docking SI, Rosengarten SD, Cook J. Achilles tendon structure improves on UTC imaging over a 5-month pre-season in elite Australian football players. *Scand J Med Sci Sports.* 2016;26(5):557-563.
13. Donoghue OA, Harrison AJ, Laxton P, Jones RK. Orthotic control of rear foot and lower limb motion during running in participants with chronic Achilles tendon injury. *Sports Biomech.* 2008;7(2):194-205.
14. Fadel PJ. Reflex control of the circulation during exercise. *Scand J Med Sci Sports.* 2015;25 Suppl 4:74-82.
15. Forst T, Hohberg C, Tarakci E, Forst S, Kann P, Pfutzner A. Reliability of lightguide spectrophotometry (O2C) for the investigation of skin tissue microvascular blood flow and tissue oxygen supply in diabetic and nondiabetic subjects. *J Diabetes Sci Technol.* 2008;2(6):1151-1156.
16. Fredberg U, Bolvig L, Lauridsen A, Stengaard-Pedersen K. Influence of acute physical activity immediately before ultrasonographic measurement of Achilles tendon thickness. *Scand J Rheumatol.* 2007;36(6):488-489.
17. Fujii N, Nikawa T, Tsuji B, Kenny GP, Kondo N, Nishiyasu T. Wearing graduated compression stockings augments cutaneous vasodilation but not sweating during exercise in the heat. *Physiol Rep.* 2017;5(9).
18. Giombini A, Dragoni S, Di Cesare A, Di Cesare M, Del Buono A, Maffulli N. Asymptomatic Achilles, patellar, and quadriceps tendinopathy: a longitudinal clinical and ultrasonographic study in elite fencers. *Scand J Med Sci Sports.* 2013;23(3):311-316.
19. Hirschmuller A, Frey V, Konstantinidis L, et al. Prognostic value of Achilles tendon Doppler sonography in asymptomatic runners. *Med Sci Sports Exerc.* 2012;44(2):199-205.
20. Ingjer F, Stromme SB. Effects of active, passive or no warm-up on the physiological response to heavy exercise. *Eur J Appl Physiol Occup Physiol.* 1979;40(4):273-282.
21. Jhingan S, Perry M, O'Driscoll G, et al. Thicker Achilles tendons are a risk factor to develop Achilles tendinopathy in elite professional soccer players. *Muscles Ligaments Tendons J.* 2011;1(2):51-56.
22. Karzis K, Kalogeris M, Mandalidis D, Geladas N, Karteroliotis K, Athanasopoulos S. The effect of foot overpronation on Achilles tendon blood supply in healthy male subjects. *Scand J Med Sci Sports.* 2016.

23. Kubo K, Ikebukuro T. Blood circulation of patellar and achilles tendons during contractions and heating. *Med Sci Sports Exerc.* 2012;44(11):2111-2117.
24. Langberg H, Olesen J, Skovgaard D, Kjaer M. Age related blood flow around the Achilles tendon during exercise in humans. *Eur J Appl Physiol.* 2001;84(3):246-248.
25. Maffulli N, Khan KM, Puddu G. Overuse tendon conditions: time to change a confusing terminology. *Arthroscopy.* 1998;14(8):840-843.
26. Magnan B, Bondi M, Pierantoni S, Samaila E. The pathogenesis of Achilles tendinopathy: a systematic review. *Foot Ankle Surg.* 2014;20(3):154-159.
27. Malliaras P, Purdam C, Maffulli N, Cook J. Temporal sequence of greyscale ultrasound changes and their relationship with neovascularity and pain in the patellar tendon. *Br J Sports Med.* 2010;44(13):944-947.
28. McAuliffe S, McCreesh K, Culloty F, Purtill H, O'Sullivan K. Can ultrasound imaging predict the development of Achilles and patellar tendinopathy? A systematic review and meta-analysis. *Br J Sports Med.* 2016;50(24):1516-1523.
29. McCrory JL, Martin DF, Lowery RB, et al. Etiologic factors associated with Achilles tendinitis in runners. *Med Sci Sports Exerc.* 1999;31(10):1374-1381.
30. McLaughlin P, Vaughan B, Shanahan J, Martin J, Linger G. Inexperienced examiners and the Foot Posture Index: A reliability study. *Man Ther.* 2016;26:238-240.
31. Michel FI, Kalin X, Metzger A, et al. [The functional sport shoe parameter "torsion" within running shoe research--a literature review]. *Sportverletz Sportschaden.* 2009;23(4):197-205.
32. Nielsen RO, Buist I, Parner ET, et al. Foot pronation is not associated with increased injury risk in novice runners wearing a neutral shoe: a 1-year prospective cohort study. *Br J Sports Med.* 2014;48(6):440-447.
33. Notarnicola A, Maccagnano G, Di Leo M, Tafuri S, Moretti B. Overload and neovascularization of Achilles tendons in young artistic and rhythmic gymnasts compared with controls: an observational study. *Musculoskelet Surg.* 2014;98(2):115-120.
34. O'Neill S, Watson PJ, Barry S. A Delphi Study of Risk Factors for Achilles Tendinopathy- Opinions of World Tendon Experts. *Int J Sports Phys Ther.* 2016;11(5):684-697.
35. Onate JA, Everhart JS, Clifton DR, Best TM, Borchers JR, Chaudhari AM. Physical Exam Risk Factors for Lower Extremity Injury in High School Athletes: A Systematic Review. *Clin J Sport Med.* 2016;26(6):435-444.
36. Ooi CC, Schneider ME, Malliaras P, et al. Sonoelastography of the Achilles Tendon: Prevalence and Prognostic Value Among Asymptomatic Elite Australian Rules Football Players. *Clin J Sport Med.* 2016;26(4):299-306.
37. Pingel J, Harrison A, Simonsen L, Suetta C, Bulow J, Langberg H. The microvascular volume of the Achilles tendon is increased in patients with tendinopathy at rest and after a 1-hour treadmill run. *Am J Sports Med.* 2013;41(10):2400-2408.
38. Priego JI, Lucas-Cuevas AG, Aparicio I, Gimenez JV, Cortell-Tormo JM, Perez-Soriano P. Long-term effects of graduated compression stockings on cardiorespiratory performance. *Biol Sport.* 2015;32(3):219-223.
39. Pufe T, Petersen WJ, Mentlein R, Tillmann BN. The role of vasculature and angiogenesis for the pathogenesis of degenerative tendons disease. *Scand J Med Sci Sports.* 2005;15(4):211-222.
40. Rahim M, El Khoury LY, Raleigh SM, et al. Human Genetic Variation, Sport and Exercise Medicine, and Achilles Tendinopathy: Role for Angiogenesis-Associated Genes. *OMICS.* 2016;20(9):520-527.
41. Ram R, Meeuwisse W, Patel C, Wiseman DA, Wiley JP. The limited effectiveness of a home-based eccentric training for treatment of Achilles tendinopathy. *Clin Invest Med.* 2013;36(4):E197-206.
42. Redmond AC, Crosbie J, Ouvrier RA. Development and validation of a novel rating system for scoring standing foot posture: the Foot Posture Index. *Clin Biomech (Bristol, Avon).* 2006;21(1):89-98.
43. Risch L, Cassel M, Mayer F. Acute effect of running exercise on physiological Achilles tendon blood flow. *Scand J Med Sci Sports.* 2017.
44. Rosengarten SD, Cook JL, Bryant AL, Cordy JT, Daffy J, Docking SI. Australian football players' Achilles tendons respond to game loads within 2 days: an ultrasound tissue characterisation (UTC) study. *Br J Sports Med.* 2015;49(3):183-187.
45. Ryan M, Grau S, Krauss I, Maiwald C, Taunton J, Horstmann T. Kinematic analysis of runners with achilles mid-portion tendinopathy. *Foot Ankle Int.* 2009;30(12):1190-1195.

46. Terada M, Wittwer AM, Gribble PA. Intra-rater and inter-rater reliability of the five image-based criteria of the foot posture index-6. *Int J Sports Phys Ther.* 2014;9(2):187-194.
47. van Dijk CN, van Sterkenburg MN, Wiegerinck JI, Karlsson J, Maffulli N. Terminology for Achilles tendon related disorders. *Knee Surg Sports Traumatol Arthrosc.* 2011;19(5):835-841.
48. van Schie HT, de Vos RJ, de Jonge S, et al. Ultrasonographic tissue characterisation of human Achilles tendons: quantification of tendon structure through a novel non-invasive approach. *Br J Sports Med.* 2010;44(16):1153-1159.
49. Venslauskas M, Ostasevicius V, Vilkinis P. Influence of low-frequency vibrations on blood flow improvement in human's limbs. *Biomed Mater Eng.* 2017;28(2):117-130.
50. Weerapong P, Hume PA, Kolt GS. The mechanisms of massage and effects on performance, muscle recovery and injury prevention. *Sports Med.* 2005;35(3):235-256.
51. Wezenbeek E, Mahieu N, Willems T, et al. What does normal tendon structure look like? New insights into tissue characterization in the Achilles tendon. *Scand J Med Sci Sports.* 2016.
52. Wezenbeek E, Willems TM, Mahieu N, Van Caekenberghe I, Witvrouw E, De Clercq D. Is Achilles tendon blood flow related to foot pronation? *Scand J Med Sci Sports.* 2017.

CHAPTER IV

Activity induced increase in Achilles tendon blood flow is age and sex dependent

Effect of age and sex on the increase in blood flow

E. Wezenbeek[1], D. De Clercq[2], N. Mahieu[1], TM. Willems[1], E. Witvrouw[1]

[1] Department of Rehabilitation Sciences and Physiotherapy, Ghent University, Ghent, Belgium,
[2] Department of Movement and Sport Sciences, Ghent University, Ghent, Belgium

Accepted for publication in American Journal of Sports Medicine
Original article (SCI 5.673, Q1) Ranking: 1/75 (Orthopedics)

Abstract

Previous research in a young adult population identified a lower increase in Achilles tendon blood flow immediately after a running activity as a significant predictor for the development of Achilles tendinopathy (AT). Furthermore, advancing age is often mentioned as a risk factor for the development of AT and the highest incidence for AT is reported to occur in middle-aged recreational male athletes. Therefore, the aim of this study was to investigate the effect of age, sex and type of physical activity on the increase in Achilles tendon blood flow. Blood flow measurements of 33 subjects aged between 18 and 25 years and 30 subjects aged between 40 and 55 years were obtained before and after four different physical activities, performed in randomized order: running, cycling, dynamic stretching, and rope skipping. Blood flow measurements of the Achilles tendon were performed before, immediately after, 5 minutes after, and 10 min after the physical activities. The effect of age, sex and physical activities on the increase in blood flow was investigated with linear mixed models. The results of this study identified that running, rope skipping and cycling resulted in a significant increase of tendon blood flow ($p \leq 0.001$), whereas stretching did not. Prominent was the finding that the increase in blood flow after activity is significantly lower in the older population compared to the younger population ($p < 0.001$). Furthermore, male participants in the older age group showed a significant lower increase in tendon blood flow compared to their female counterparts ($p = 0.019$). This study identified that both sex and age significantly influence the increase in blood flow after activity, possibly explaining the increased risk for AT in middle-aged recreational athletes. This study possibly identified one of the mechanisms why an older male population is at increased risk for developing AT. Since the lower increase in blood flow is identified as a risk factor in previous research, preventative measures should focus on improving this blood flow during physical activity in the physically active older male population.

Key terms: Achilles tendon, Blood flow, Physical activity, Sex, Age

Introduction

Although the Achilles tendon is the largest and strongest tendon in the human body, injuries are common.[37] The most common Achilles tendon injury is tendinopathy.[4] The highest incidence for Achilles tendinopathy (AT) is usually reported to occur in middle-age recreational male athletes.[2, 26, 33] This is surprising since the amount of sports participation decreases with advancing age and most other sports injuries are implied to occur in a younger age group.[39] Therefore, advancing age is often mentioned as a risk factor for the development of AT.[12, 25, 26, 44] Advancing age is commonly associated with increased prevalence of degenerative changes in tendons, such as decreased cellularity, lack of fiber organization, and decreased vascularity.[3, 26, 31] It is suggested that in a hypovascular tendon the extent of necessary matrix remodeling is restricted, which may lead to weakening of the tendon structure, causing tendinopathy.[35, 36]

An increase in Achilles tendon blood flow is a normal physiological response to activity since physical activity generates an increased metabolic demand. In order to fulfill this increased metabolic demand a necessary cardiovascular redistribution of blood flow to the working soft tissue is induced.[15] An impaired exercise induced transient increase in blood flow leads to inadequate tissue oxygen delivery and metabolic dysregulation.[14, 20] This relates to AT since previous research in an adolescent population identified a lower increase in blood flow immediately after a running activity as a significant predictor for the development of AT.[42]

Since the incidence of tendinopathy increases with age, and a lower increase in blood flow immediately after activity is identified as a risk factor in an adolescent population, it is possible that this increase in blood flow after activity is altered in an older population. However, it is striking that, to date, no research has investigated whether this blood flow response after activity is age-, sex- or activity-dependent. Therefore the aim of this study was to investigate the effect of age and sex on the Achilles tendon blood flow after different physical activities.

Materials and methods

This study was performed at Ghent University Hospital, Belgium. Approval was obtained by the Ethics Committee of the Ghent University Hospital (number of approval: EC/2013/401) and all participants signed an informed consent. This study was registered at ClinicalTrials.org PRS with the registration number 2013/0401-NCT03218605. Participants were subdivided into the younger or older age group.

Multiple physical activities were performed on different days in randomized order to prevent fatigue and a possible interaction between activities: running, cycling, dynamic stretching, and rope skipping. Blood flow measurements of the dominant Achilles tendon were performed before, immediately after, 5 minutes after, and 10 min after the physical activities.

Participants

In this study, 33 subjects aged between 18 and 25 years and 30 subjects aged between 40 and 55 years participated. Participants were excluded from this study if they had a history of AT, surgery of the lower extremity or severe trauma in the lower extremity (LE) within the previous year. All participants performed at least 1 hour of weekly sports participation and did not participate in more than 3 training sessions a week. This resulted in 63 included participants (31 male and 32 female), with a mean age of 34.4 years and an average weekly sports participation of 2.6 hours (Table 1).

Study design

Participants were instructed not to engage in any sports activities 48 h prior to testing to ensure a valid measure of exercise related increase in blood flow. Before the physical activity was performed, participants were instructed to rest on a treatment table for 10 minutes. After this resting period, blood flow was measured for a first time in prone position. Then, participants performed a physical activity (running, cycling, dynamic stretching or rope skipping) in randomized order for 10 minutes. Immediately after this, blood flow measurements were repeated in the same prone position on the treatment table (immediately post activity blood flow). The treatment table was positioned next to the area where the physical activities were performed to minimalize the transition time. Next, participants were asked to rest on the treatment table, while blood flow measurements were repeated 5 minutes after the physical activity (5 min post blood flow). In addition, in the older population, the blood flow was also registered 10 minutes after the physical activity (10 min post blood flow). As a consequence, three blood flow measurements were conducted per physical activity in both the younger and the older population: pre-activity blood flow, immediate post-activity blood flow and 5 min post-activity blood flow, and additionally 10 min post-activity blood flow was registered only in the older population. Also heart rate and Rate of Perceived Exertion (RPE) were measured. This test protocol was repeated for all physical activities, with minimum 48 hours and maximum one week between two physical activities. Participants performed all physical activities shod, wearing self-selected running shoes. Before the experimental protocol, participants were asked to fill in a questionnaire concerning demographic and anthropometric data (age, sex, height, weight, limb dominance), physical activities, and injury history.

Blood flow measurement

Blood flow data at the midportion of the dominant Achilles tendon were collected by use of the oxygen-to-see® (O2C) device (LEA Medizintechnik Giessen, Germany). This non-invasive device is capable of measuring the perfusion and oxygenation of the subcutaneous tissue using an optical flat fiber probe. Measurements were performed as described in Wezenbeek et al.[43] at a depth of 8mm. The head of the probe was placed flat on the tissue at the measurement point, without pressure or movement. For standardization purposes, measurements were performed in a darkened room with constant temperature. The measurement has been shown to be reliable and valid.[18, 43]

Physical activities

All activities were performed in controlled laboratory conditions (19–21 °C; 65%rH), to exclude environmental influences. The younger and older population both performed dynamic stretching, running and rope skipping, and additionally the older population also performed cycling. These activities were performed in randomized order and every activity was performed with a duration of 10 minutes. Heart rate was objectively measured with a heart rate monitor (cfr. Polar©) and registered pre-, immediately post and five minutes post- activity in all participants, and also 10 minutes post-activity in the older population. The intensity of the exercise was subjectively monitored with a Rate of Perceived Exertion (RPE) scale immediately post-activity in all participants.

Running

Participants were instructed to run on a motorized treadmill (HP Cosmos Saturn, Traunstein, Germany) at a running pace of 2.7m/s, without an inclination angle.[8, 22]

Cycling

Participants were placed on a bicycle ergometer (Ergofit cycle 400 Gymna, Bilzen, Belgium) with standard pedals, and the pedal bars were positioned under the heads of the metatarsal bones. Participants were instructed to remain seated and to generate 90 revolutions per minute (rpm) to increase the muscle activity of Gastrocnemius and Soleus.[16] Power output was determined at 100 watt.[29]

Rope skipping

Ninety seconds of rope skipping were alternated with 30 seconds of rest, until the total duration of ten minutes was completed. The length of the rope was individually adjusted: from the ground to the axilla of the subject.

Dynamic stretching

During the dynamic stretch of the calf muscles of the dominant leg, the participant performed a classic standing wall push of the non-dominant leg. The participants were instructed that the endpoint of the stretch should be at the point just before discomfort, and that this self-chosen distance should be reached while the dominant knee remained straight and the dominant heel remained on the floor. Once the participants had reached this stretching position, they were instructed to move up and down at a pace of one movement per second with the front (non-dominant) knee.[27]

Statistical analysis

Statistical analysis was done with the SPSS V.24 Statistical Software package (IBM Corp., New York, USA). The effect of age, sex and physical activities (running, cycling, dynamic stretching or rope skipping) on the increase in blood flow immediately post-, 5 min post-, and 10 min post-activity (immediately post-, 5 min post-, 10 min post-activity blood flow minus pre-activity blood flow) was investigated with linear mixed models. Analyses were executed with participants as a random factor, and age, sex and physical activities as fixed predictors. Post hoc analyses were performed using Bonferroni corrections. The effect of possible covariates as BMI, heart rate, RPE and sports participation was investigated. The residuals of the linear mixed model were checked for normal distribution and homoscedasticity. The level of significance was set at $\alpha=0.05$.

Results

BMI, heart rate, RPE and sports participation did not have an effect on the outcome parameter, and are therefore not included as covariates in the analyses. Subject demographics can be found in Table 1.

Table 2 displays the values of the blood flow variables, expressed in arbitrary units (AU), per activity and timing of measurement for the younger and older group. In both age categories no significant differences in pre-activity flow were found between the different activities ($p=0.077$ and $p=0.484$, for the younger and older age group respectively). The immediately post flow was significantly higher compared to the pre-activity flow after running, rope skipping and cycling ($p<0.001$ for both the younger and older age group after running and rope skipping, and $p=0.001$ for the older age group after cycling). The tendon blood flow measured 5 and 10 minutes after running, rope skipping and cycling was significantly higher than the pre-activity flow ($p<0.002$ for 5 and 10 minutes after running and rope

Table 1 Subject demographics and tendon characteristics for the younger and older group.

	Total Group (n = 63)	Younger Group (n=33)	Older Group (n=30)
Age, y	34,4 ± 13.7	21.9 ± 1.7	48.2 ± 4.9
Height, m	1.74 ± 0.09	1.76 ± 0.09	1.73 ± 0.08
Weight, kg	69.9 ± 11.7	67.0 ± 8.6	73.2 ± 13.7
Body Mass Index, kg/m^2	22.9 ± 2.9	21.6 ± 1.8	24.4 ± 3.2
Sport participation, hours/week	2.6 ± 1.2	3.2 ± 1.7	2.1 ± 1.0
Sex, n (%)			
Males	31/63 (49)	16/33 (48)	15/30 (50)
Females	32/63 (51)	17/33 (52)	15/30 (50)
Limb dominance, n (%)			
Right dominant	55/63 (87)	31/33 (94)	24/30 (80)
Left dominant	8/63 (13)	2/33 (6)	6/30 (20)

[a] Data expressed as mean ± SD or n (%).

skipping in both age categories, and p= 0.043 and p=0.030 for 5 and 10 minutes after cycling in the older age category), but significantly lowered compared to the flow immediately post -activity (p<0.001 for 5 and 10 minutes after running and rope skipping in both age categories, and p= 0.023 and p=0.025 for 5 and 10 minutes after cycling in the older age category). No significant differences were found between the flow 5 minutes post-activity and the flow 10 minutes post activity for any activity in the older age category (p =0.499). Dynamic stretching did not significantly increase the blood flow immediately after activity, nor after 5 or 10 minutes (p-values range between 0.277 and 0.566 for the younger and older age group).

Tendon flow characteristics are displayed in Figure 1. In both age categories the increase in blood flow (immediately post – pre activity) is significantly higher in rope skipping and running compared to the other activities (p<0.001). Analyses between the age categories showed that the increase in blood flow (immediately post – pre activity) is significantly smaller in the older age group compared to the younger age group, as illustrated in Figure 2 (p<0.001 for running and rope skipping). Also, the decrease after activity (5 min post - immediately post) is significantly smaller in the older age group compared to the younger age group (p<0.001 for running and rope skipping). The increase in blood flow 5 minutes after activity (5 min post activity – pre activity) showed no significant difference between the age categories (p=0.333 for running and rope skipping).

Table 2 Overview of tendon flow characteristics per activity and timing of measurement for the younger and older group per activity.

Activity	Timing	Younger group Mean (AU) ± SD	Older group Mean (AU) ± SD
Dynamic stretching	Pre - activity	57,4 ± 16,7	64,4 ± 15,0
	Immediately post -activity	59,3 ± 20,0	67,2 ± 17,4
	5 min post -activity	56,1 ± 15,3	66,6 ± 16,8
	10 min post -activity	/	66,4 ± 18,7
Rope skipping	Pre - activity	51,5 ± 14,8	64,2 ± 16,3
	Immediately post -activity	134,1 ± 48,0	114,9 ± 46,4
	5 min post -activity	72,5 ± 23,4	85,6 ± 36,3
	10 min post -activity	/	84,8 ± 24,7
Cycling	Pre - activity	/	65,3 ± 23,7
	Immediately post -activity	/	81,4 ± 31,6
	5 min post -activity	/	71,5 ± 22,3
	10 min post -activity	/	73,5 ± 21,8
Running	Pre - activity	48,5 ± 10,6	70,4 ± 19,2
	Immediately post -activity	141,6 ± 48,1	119,2 ± 42,4
	5 min post -activity	76,6 ± 24,8	88,7 ± 26,7
	10 min post -activity	/	84,5 ± 27,1

Indicates p-value < 0.05

Table 3 shows the values of the RPE and heart rate, expressed in bpm, per activity and timing of measurement for the younger and older group. In both age categories no significant differences in pre-activity heart rate were found between the different activities (p=0.314 and p=0.122, for the younger and older age group respectively). The immediately post-activity heart rate was significantly higher compared to the pre-activity heart rate after all the performed activities (p<0.001 in both age categories). The heart rate measured 5 and 10 minutes post–activity was significantly lower compared to the immediately post-activity heart rate after all the performed activities (p<0.001 in both age categories). In both age categories the increase in heart rate (immediately post – pre activity) is significantly higher in rope skipping and running compared to the other activities (p<0.001). Analyses between the age categories showed no significant differences in increase in heart rate (immediately post – pre activity) (p=0.180 for running and rope skipping). Also the decrease in heart rate after activity (5 min post - immediately post) showed no significant differences between the 2 age categories (p=0.176 for running and rope skipping).

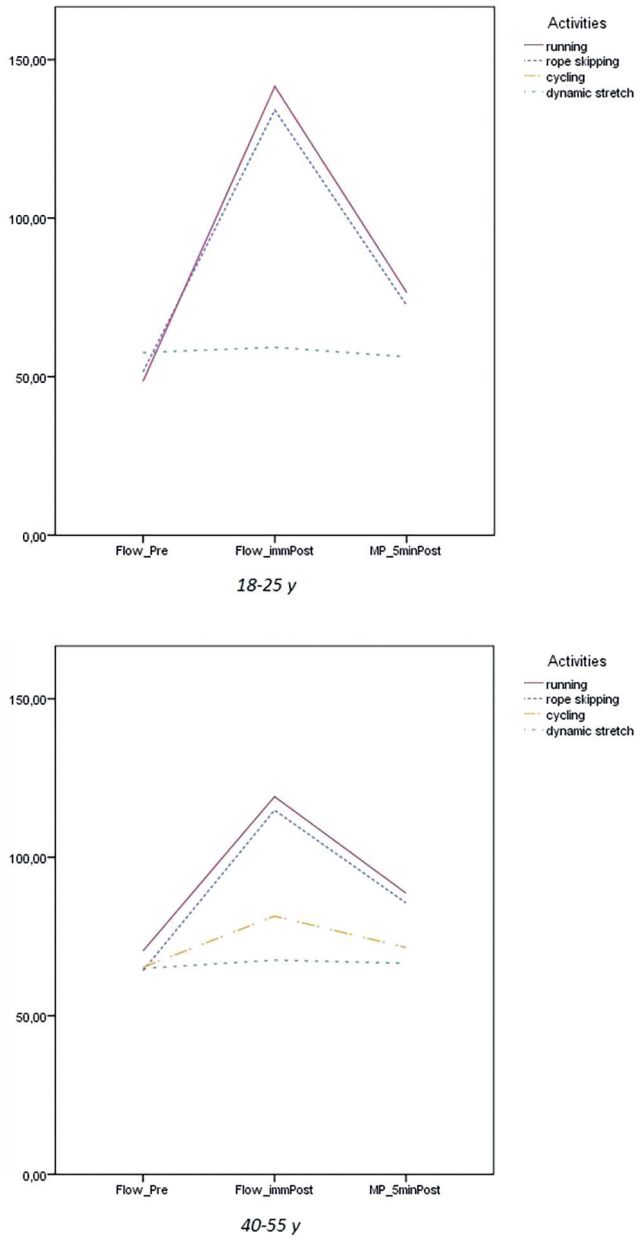


Fig. 1 Overview of tendon flow (AU) per activity and timing of measurement for the younger and older age group.

Table 3 Overview of heart rate and RPE per activity and timing of measurement for the younger and older group per activity

Activity	Timing	Younger group		Older group	
		RPE	HR	RPE	HR
Dynamic stretching	Pre - activity	/	74.1 ± 9.5	/	67.5 ± 10.0
	Immediately post -activity	9.8 ± 2.2	96.3 ± 16.0	7.9 ± 1.6	79.5 ± 10.9
	5 min post -activity	/	75.5 ± 10.0	/	65.1 ± 8.4
	10 min post -activity	/	/	/	63.2 ± 8.6
Rope skipping	Pre - activity	/	76.6 ± 12.5	/	69.3 ± 10.0
	Immediately post -activity	14.8 ± 2.1	161.1 ±18.9	14.9 ±2.0	156.2 ± 14.5
	5 min post -activity	/	98.2 ± 10.3	/	95.2 ± 9.9
	10 min post -activity	/	/	/	87.6 ± 11.5
Cycling	Pre - activity	/	/	/	68.7 ± 10.6
	Immediately post -activity	/	/	11.4 ±2.6	127.6 ± 24.0
	5 min post -activity	/	/	/	80.3 ± 15.8
	10 min post -activity	/	/	/	75.7 ± 13.5
Running	Pre - activity	/	72.7 ± 9.7	/	72.2 ± 9.8
	Immediately post -activity	13.6 ± 2.3	149.1 ±24.2	13.7 ±1.9	156.9 ± 20. 8
	5 min post -activity	/	94.1 ± 11.2	/	90.8 ± 11.9
	10 min post -activity	/	/	/	85.8 ± 11.1

Indicates p-value < 0.05

BMI, heart rate, RPE and amount of sports participation did not have a significant effect on the increase in tendon blood after activity and were therefore not included as covariates in the analyses. Sex did significantly influence the increase in tendon blood flow after activity, but only in the older population (p=0.019). Figure 2 demonstrates the increase in tendon blood flow (immediately post – pre activity) after rope skipping and running per age category for male and female participants. As illustrated, no significant difference in increase in blood flow was found between males and females in our young population, whereas the male participants in the older population showed a significant lower increase in tendon blood compared to the female participants.

The effects of physical activities, age categories and sex on the increase in blood flow after activity (immediately post - pre-activity blood flow) are presented in Table 4.

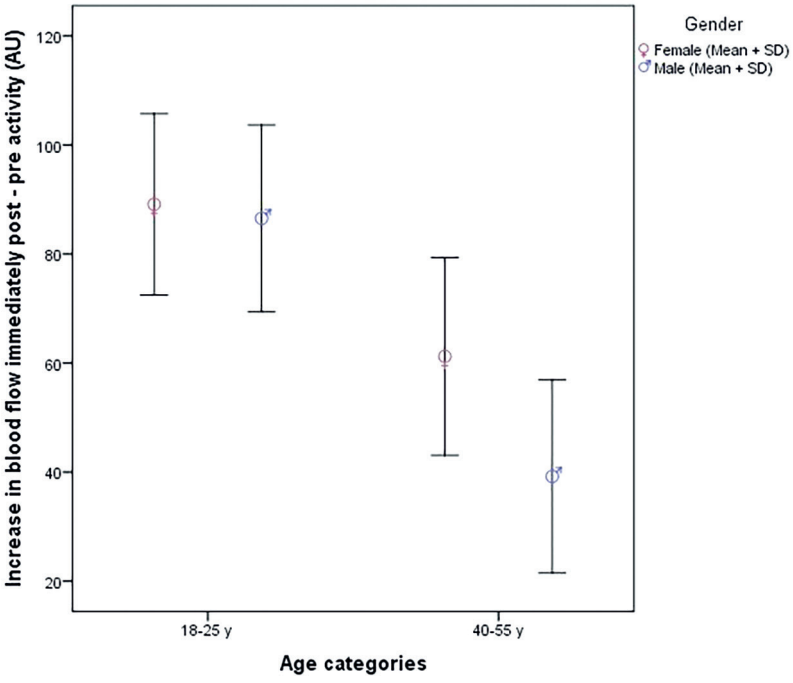


Fig. 2 The increase in blood flow per age category.

Table 4 Effect of age categories, activities and sex on the increase in blood flow after activity

	b	SE b	P-value	95% Confidence Interval	
				Lower Bound	Upper Bound
Intercept	54,50	6,07	<0,001	42,48	66,51
Age (older age is reference category)					
Age=18-25y	24,36	5,72	**<0,001***	12,95	35,77
Activities (running is reference category)					
Dynamic stretch	-70,55	6,07	**<0,001***	-82,55	-58,55
Rope skipping	-5,26	6,05	0,385	-17,21	6,68
Cycling	-44,06	7,90	**<0,001***	-59,68	-28,44
Sex (male is reference category)					
Female sex	10,67	5,41	**0,054**	-0,18	21,51

*indicates p-value < 0.05

Discussion

The results of this study identify that running, rope skipping and cycling resulted in a significant increase of tendon blood flow. Prominent was the finding that the increase in blood flow after activity is significantly lower in the older population compared to the younger population. Furthermore, male participants in the older age group showed a significant lower increase in tendon blood flow compared to their female counterparts.

Effect of age on the Achilles tendon blood flow

The results of this study show that the increase in blood flow after activity is lower in the older population compared to the younger age group. This is an important finding since previous research showed that the lower the increase in blood flow after physical activity, the higher the hazard for developing AT.[42] Therefore, this lower increase in blood flow after activity observed in an older population places this population at higher risk for the development of AT, possibly explaining the higher incidence of AT in middle-aged athletes.

It is well established that physical activity generates an increased metabolic demand. In order to fulfill this increased metabolic demand a necessary cardiovascular redistribution of blood flow to the working soft tissue is induced.[15] Consequently, an increase in Achilles tendon blood flow as a normal physiological response to activity has been observed.[34] This exercise induced transient increase in blood flow is primarily due to a local vasodilatory response that serves as a feedforward mechanism for exercise hyperemia.[13] With advancing age, the normal regulation of exercising blood flow is found to be impaired in the skeletal muscles. The age-associated impairments in vascular conductance are thought to be the result of vascular stiffness and impaired local vasodilatory or vasoconstrictor signaling.[20] This impaired exercising muscle blood flow leads to inadequate tissue oxygen delivery and metabolic dysregulation.[14, 20] This is important since metabolic disorders are stated to be of paramount relevance to the progression of tendon damage.[1]

The results of this study demonstrate that the increase in blood flow after activity is lower in the older population compared to the younger age group. This is in accordance with the study of Langberg et al.,[24] who found a non-significantly lower peritendinous absolute flow in the middle-aged population compared to a younger age group after a static calf muscle exercise. Previous research in the Achilles tendon found an association between a decrease in microcirculation and the onset of tendon degeneration.[17] Furthermore, this decreased vascularity is thought to cause decreased tensile strength, or to indirectly weaken the tendon through degenerative changes and structural vulnerability with decreased healing potential.[28, 36, 40] Therefore, it is plausible that this age-induced lower increase in blood flow after activity and possibly resultant altering of metabolic properties, leads to weakening of the normal tendon structure and eventually AT.

Clinical consequences are that this study possibly identified one of the mechanisms why an older male population is at increased risk for developing AT. Since the lower increase in blood flow is identified as a risk factor in previous research, it seems logical that the increase in blood flow during activity should be stimulated in an older population. Previous research stated that prostaglandins, nitric oxide, heat, massage, vibration techniques and longer warm-up duration elevate the blood flow. However, further research is needed to investigate the vascular response of these latter techniques in the Achilles tendon since current literature is mainly limited to changes in blood flow in the skin surface. [42] In addition, further research should focus on investigating strategies to modify this diminished vascular response to activity in the Achilles tendon. The effect of training programs on the vascular response to activity in tendons should be investigated since it is stated that physical

activity has the potential to offset the age-related decline in blood flow in skeletal muscles during exercise, thereby improving oxygen delivery and an enhanced energy production from oxidative metabolism. This effect has been showed in skeletal muscles after a period of aerobic high-intensity exercise training,[6, 32] but to the best of our knowledge, this has not yet been studied in tendons.

Effect of sex on the Achilles tendon blood flow

Next, this study found that male participants in the older population group showed a lower increase in tendon blood flow compared to the females in this age group. The lower increase in tendon flow after activity in the male participants in the older population are in agreement with previous research stating that male sex and advancing age are associated with diminished tendon blood flow. This was investigated in seventy-five participants using laser Doppler flowmetry in the assessment of Achilles tendon blood flow.[5] Furthermore, previous research studied the effect of estrogen on the peripheral blood flow, and found a significant increase in blood flow associated with a reduction of vascular resistance, explaining some of the beneficial effects of estrogen on the vascular system.[41] The results of this study might possible explain the higher incidence of AT found in male middle-aged athletes.[5, 26] However, it should be considered that differences in physical activity between males and females make it difficult to evaluate the independent effect of sex on the Achilles tendon blood flow, since no previous study investigated sex as an independent risk factor per amount of sports participation (e.g. 1000 hours). As clinical implication, it seems logical that middle-aged men with a lower increase in blood flow after physical activity need more emphasize on warm-up in order to improve the vascular response to activity and consequently reduce the risk for developing AT.

Effect of activities on the Achilles tendon blood flow

Running, rope skipping and cycling resulted in an increase of tendon blood flow, and this increase after running and rope skipping was significantly higher compared to cycling. In addition, the results of this study identified that dynamic stretching did not significantly alter the Achilles tendon blood flow. An explanation for the induced increase in tendon blood flow after running, rope skipping, and cycling is that these activities are dynamic exercises that require contraction and relaxation of the working tissue. This is directly related to physiological processes, such as an increase in body temperature and an increase in the metabolic rate of the working tissue, that leads to vasodilation of the blood vessels in the working soft tissues, subsequently leading to an increase in blood flow.[11, 15, 19, 21, 23, 34, 38] The lower increase in blood flow observed after cycling compared to rope skipping and running is not surprising and in agreement with previous research observing a

lower total limb blood flow immediately post-exercise in cycling compared to running. Millet et al.[30] explained this by differences in muscle pump efficiency, exercise position, and differences in type of muscle contraction between running and cycling. Furthermore, during exercise skeletal muscle blood flow is shown to increase with increased work load [9] and the work load during running is higher compared to cycling.[7]

Methodological considerations

Several limitations of this study are noteworthy. First, our study was performed on a healthy population. Therefore, our results cannot be generalized to pathological tendons. Next, the study design of the older population was more extensive since cycling and the blood flow measurement 10 minutes after activity was added to the protocol used in the younger population. Since this younger population was tested first, preliminary analysis showed a decrease in blood flow 5 minutes after physical activity but the further course of the blood flow remained unclear. In addition, cycling was added for the comparison of the vascular response to a non-weight bearing physical activity with working muscle pump to be able to interpret the results more profound. Therefore, the cycling activity and the measurement 10 min after physical activity were added to the study design of the later tested older age group. We are aware that this is a limitation of this study. Nevertheless, we believe that adding cycling and the extra blood flow measurement ensured a more correct and more extensive interpretation and discussion of the results. Also, since an association between the increase in Achilles tendon blood flow and tendon degeneration has been shown, measuring tendon structure would have given this study an added value. Another limitation of this study is that no US imaging of the tendon nor the detection of possibly present neovascularization was performed to exclude/include participants in this study. In this study, we have used the oxygen-to-see to perform the blood flow measurements. There is no gold standard for measuring blood flow,[10] and this non-invasive device has shown to be valid and reliable.[18, 43]

Despite several limitations, this study is the first to investigate the effect of age and sex on the Achilles tendon blood flow after different physical activities performed in randomized order.

Conclusion

This study is the first to investigate the effect of age and sex on the Achilles tendon blood flow after different physical activities. The results of this study showed that the increase in Achilles tendon blood flow during physical activity is lower in an older population, and that the male participants in the older population showed a lower increase in tendon blood flow compared to their females counterparts. Furthermore, the increase in Achilles tendon blood flow was activity-dependent, with running and rope skipping resulting in the highest increase in blood flow, followed by cycling. Stretching did not alter the Achilles tendon blood flow.

Addendum

Table 2 Overview of tendon flow characteristics per activity and timing of measurement for the younger and older group per activity – only the male participants

Activity	Timing	Younger group Mean (AU) ± SD	Older group Mean (AU) ± SD
Dynamic stretching	Pre - activity	60,3 ± 19,6	62,8 ± 16,7
	Immediately post -activity	60,2 ± 24,3	62,0 ± 15,6
	5 min post -activity	56,0 ± 13,8	63,9 ± 12,8
	10 min post -activity	/	62,9 ± 18,9
Rope skipping	Pre - activity	58,6 ± 13,7	60,4 ± 17,9
	Immediately post -activity	148,8 ± 49,7	105,2 ± 45,3
	5 min post -activity	76,9 ± 18,7	80,1 ± 22,3
	10 min post -activity	/	84,8 ± 19,9
Cycling	Pre - activity	/	58,9 ± 15,0
	Immediately post -activity	/	66,2 ± 17,5
	5 min post -activity	/	68,1 ± 18,0
	10 min post -activity	/	67,9 ± 18,6
Running	Pre - activity	50,0 ± 11,1	69,6 ± 15,3
	Immediately post -activity	132,8 ± 60,7	103,1 ± 31,1
	5 min post -activity	80,0 ± 27,0	81,8 ± 14,6
	10 min post -activity	/	76,7 ± 14,7

Indicates p-value < 0.05

References

1. Abate M, Schiavone C, Salini V, Andia I. Occurrence of tendon pathologies in metabolic disorders. *Rheumatology (Oxford).* 2013;52(4):599-608.
2. Alfredson H, Lorentzon R. Intratendinous glutamate levels and eccentric training in chronic Achilles tendinosis: a prospective study using microdialysis technique. *Knee Surg Sports Traumatol Arthrosc.* 2003;11(3):196-199.
3. Ames PR, Longo UG, Denaro V, Maffulli N. Achilles tendon problems: not just an orthopaedic issue. *Disabil Rehabil.* 2008;30(20-22):1646-1650.
4. Asplund CA, Best TM. Achilles tendon disorders. *BMJ.* 2013;346:f1262.
5. Astrom M. Laser Doppler flowmetry in the assessment of tendon blood flow. *Scand J Med Sci Sports.* 2000;10(6):365-367.
6. Beere PA, Russell SD, Morey MC, Kitzman DW, Higginbotham MB. Aerobic exercise training can reverse age-related peripheral circulatory changes in healthy older men. *Circulation.* 1999;100(10):1085-1094.
7. Bijker KE, de Groot G, Hollander AP. Differences in leg muscle activity during running and cycling in humans. *Eur J Appl Physiol.* 2002;87(6):556-561.
8. Billat VL, Richard R, Binsse VM, Koralsztein JP, Haouzi P. The V(O2) slow component for severe exercise depends on type of exercise and is not correlated with time to fatigue. *J Appl Physiol (1985).* 1998;85(6):2118-2124.
9. Bonnelykke Sorensen V, Wroblewski H, Galatius S, Haunso S, Kastrup J. Assessment of continuous skeletal muscle blood flow during exercise in humans. *Microvasc Res.* 2000;59(2):301-309.
10. Casey DP, Curry TB, Joyner MJ. Measuring muscle blood flow: a key link between systemic and regional metabolism. *Curr Opin Clin Nutr Metab Care.* 2008;11(5):580-586.
11. Clifford PS. Skeletal muscle vasodilatation at the onset of exercise. *J Physiol.* 2007;583(Pt 3):825-833.
12. Cook JL, Khan KM, Purdam C. Achilles tendinopathy. *Man Ther.* 2002;7(3):121-130.
13. Crecelius AR, Kirby BS, Luckasen GJ, Larson DG, Dinenno FA. Mechanisms of rapid vasodilation after a brief contraction in human skeletal muscle. *Am J Physiol Heart Circ Physiol.* 2013;305(1):H29-40.
14. DeLorey DS, Kowalchuk JM, Paterson DH. Effect of age on O(2) uptake kinetics and the adaptation of muscle deoxygenation at the onset of moderate-intensity cycling exercise. *J Appl Physiol (1985).* 2004;97(1):165-172.
15. Fadel PJ. Reflex control of the circulation during exercise. *Scand J Med Sci Sports.* 2015;25 Suppl 4:74-82.
16. Faria EW, Parker DL, Faria IE. The science of cycling: physiology and training - part 1. *Sports Med.* 2005;35(4):285-312.
17. Fenwick SA, Curry V, Harrall RL, Hazleman BL, Hackney R, Riley GP. Expression of transforming growth factor-beta isoforms and their receptors in chronic tendinosis. *J Anat.* 2001;199(Pt 3):231-240.
18. Forst T, Hohberg C, Tarakci E, Forst S, Kann P, Pfutzner A. Reliability of lightguide spectrophotometry (O2C) for the investigation of skin tissue microvascular blood flow and tissue oxygen supply in diabetic and nondiabetic subjects. *J Diabetes Sci Technol.* 2008;2(6):1151-1156.
19. Gonzalez-Alonso J, Calbet JA, Boushel R, et al. Blood temperature and perfusion to exercising and non-exercising human limbs. *Exp Physiol.* 2015;100(10):1118-1131.
20. Hearon CM, Jr., Dinenno FA. Regulation of skeletal muscle blood flow during exercise in ageing humans. *J Physiol.* 2016;594(8):2261-2273.
21. Hong KS, Kim K. Skeletal muscle contraction-induced vasodilation in the microcirculation. *J Exerc Rehabil.* 2017;13(5):502-507.
22. Keir DA, Zory R, Boudreau-Lariviere C, Serresse O. Mechanical efficiency of treadmill running exercise: effect of anaerobic-energy contribution at various speeds. *Int J Sports Physiol Perform.* 2012;7(4):382-389.
23. Kjaer M, Langberg H, Miller BF, et al. Metabolic activity and collagen turnover in human tendon in response to physical activity. *J Musculoskelet Neuronal Interact.* 2005;5(1):41-52.
24. Langberg H, Olesen J, Skovgaard D, Kjaer M. Age related blood flow around the Achilles tendon during exercise in humans. *Eur J Appl Physiol.* 2001;84(3):246-248.
25. Maffulli N, Wong J, Almekinders LC. Types and epidemiology of tendinopathy. *Clin Sports Med.* 2003;22(4):675-692.

26. Magnan B, Bondi M, Pierantoni S, Samaila E. The pathogenesis of Achilles tendinopathy: a systematic review. *Foot Ankle Surg.* 2014;20(3):154-159.
27. Mahieu NN, McNair P, De Muynck M, et al. Effect of static and ballistic stretching on the muscle-tendon tissue properties. *Med Sci Sports Exerc.* 2007;39(3):494-501.
28. Marquez-Arabia WH, Gomez-Hoyos J, Gomez M, et al. Influence of Aging on Microvascular Supply of the Gluteus Medius Tendon: A Cadaveric and Histologic Study. *Arthroscopy.* 2017;33(7):1354-1360.
29. Marsh AP, Martin PE, Foley KO. Effect of cadence, cycling experience, and aerobic power on delta efficiency during cycling. *Med Sci Sports Exerc.* 2000;32(9):1630-1634.
30. Millet GP, Vleck VE, Bentley DJ. Physiological differences between cycling and running: lessons from triathletes. *Sports Med.* 2009;39(3):179-206.
31. Moller A, Astron M, Westlin N. Increasing incidence of Achilles tendon rupture. *Acta Orthop Scand.* 1996;67(5):479-481.
32. Nyberg M, Gliemann L, Hellsten Y. Vascular function in health, hypertension, and diabetes: effect of physical activity on skeletal muscle microcirculation. *Scand J Med Sci Sports.* 2015;25 Suppl 4:60-73.
33. O'Neill S, Watson PJ, Barry S. A Delphi Study of Risk Factors for Achilles Tendinopathy- Opinions of World Tendon Experts. *Int J Sports Phys Ther.* 2016;11(5):684-697.
34. Pingel J, Harrison A, Simonsen L, Suetta C, Bulow J, Langberg H. The microvascular volume of the Achilles tendon is increased in patients with tendinopathy at rest and after a 1-hour treadmill run. *Am J Sports Med.* 2013;41(10):2400-2408.
35. Pufe T, Petersen WJ, Mentlein R, Tillmann BN. The role of vasculature and angiogenesis for the pathogenesis of degenerative tendons disease. *Scand J Med Sci Sports.* 2005;15(4):211-222.
36. Rahim M, El Khoury LY, Raleigh SM, et al. Human Genetic Variation, Sport and Exercise Medicine, and Achilles Tendinopathy: Role for Angiogenesis-Associated Genes. *OMICS.* 2016;20(9):520-527.
37. Ram R, Meeuwisse W, Patel C, Wiseman DA, Wiley JP. The limited effectiveness of a home-based eccentric training for treatment of Achilles tendinopathy. *Clin Invest Med.* 2013;36(4):E197-206.
38. Roseguini BT, Davis MJ, Harold Laughlin M. Rapid vasodilation in isolated skeletal muscle arterioles: impact of branch order. *Microcirculation.* 2010;17(2):83-93.
39. Sobrino FJ, Guillen P. Overuse Injuries in Professional Ballet: Influence of Age and Years of Professional Practice. *Orthop J Sports Med.* 2017;5(6):2325967117712704.
40. Theobald P, Benjamin M, Nokes L, Pugh N. Review of the vascularisation of the human Achilles tendon. *Injury.* 2005;36(11):1267-1272.
41. Volterrani M, Rosano G, Coats A, Beale C, Collins P. Estrogen acutely increases peripheral blood flow in postmenopausal women. *Am J Med.* 1995;99(2):119-122.
42. Wezenbeek E, Willems T, Mahieu N, et al. The Role of the Vascular and Structural Response to Activity in the Development of Achilles Tendinopathy: A Prospective Study. *Am J Sports Med.* 2018:363546517750854.
43. Wezenbeek E, Willems TM, Mahieu N, Van Caekenberghe I, Witvrouw E, De Clercq D. Is Achilles tendon blood flow related to foot pronation? *Scand J Med Sci Sports.* 2017.
44. Wise BL, Peloquin C, Choi H, Lane NE, Zhang Y. Impact of age, sex, obesity, and steroid use on quinolone-associated tendon disorders. *Am J Med.* 2012;125(12):1228 e1223-1228 e1228.

General discussion

Summary

All of the studies embedded within this dissertation aimed to better understand demographic, structural, biomechanical and vascular parameters of AT and to prospectively investigate suggested risk factors for the development of AT. A summary of the research findings per chapter are presented below.

Before being able to establish aberrant tendon structure with UTC, normative data are imperative. Therefore, in chapter I, the normal tendon tissue characterisation was described. The Achilles tendons of active, healthy adolescents contained high levels of echo-types I and II, representing intact and aligned tendon bundles (echo-type I) and discontinuous or waving tendon bundles (echo-type II). This indicated that the presence of echo-type II tendon bundles can be considered as normal in active adolescents. Furthermore, our results highlight differences in the UTC echopattern in healthy Achilles tendons based on sex. More specifically, female tendons contained more echo-type II compared with male tendons.

The underlying mechanism of the whipping hypothesis was investigated in chapter II. This hypothesis suggests that (excessive) pronation plays an important role in the development of AT since pronation is thought to create vascular blanching of the midportion of the Achilles tendon. Evidence was provided to support the suggested mechanism of this whipping hypothesis, since more eversion excursion during running was associated with a significant lower increase in blood flow after running. In chapter III, several suggested intrinsic risk factors were prospectively examined to investigate their role in the development of AT. A lower increase in blood flow after running was identified as a significant risk factor for the development of AT. Also, in this adolescent population, females were more at risk to develop AT in comparison with males, when performing a similar sports program. Foot posture as such was not identified as a risk factor. A general ultrasound tissue characterisation evaluation or measuring tendon thickness was not suited as early detection method for AT.

Chapter IV examined the effect of demographics on the vascular response to activity. The results indicated that the vascular response to activity is lower in the older population compared to the younger population. Moreover, a sex-related difference was seen in this older population. More specifically, male participants in the older age group showed a lower vascular response to activity compared with their female counterparts.

Main discussion

What is the importance of tendon structure in the underlying mechanism of AT and could this be established as a risk factor for AT?

1. How does a normal tendon look like in an active adolescent population?
Healthy tendons of a young mature population were studied in chapter I. A fairly high percentage of echo-type II fibers, indicating disorganized and waving tendon bundles, was observed in this population. This finding was unexpected since an increase in echo-type II has been suggested to negatively affect the normal tendon integrity.[72, 80] This high percentage of echo-type II fibers can be interpreted as a remodeling of the tendon. Indeed, Rosengarten et al.[72] stated that echo-type II is indicative for reversible tendon matrix remodeling. Furthermore, tissue turnover, indicating tendon adaptability, is mostly seen during adolescence.[35, 61] Therefore, it is possible that the presence of echo-type II in this healthy adolescent population, is a positive/adaptive response to load, rather than a negative/early pathological response from the tendon. The presence of echo-types III and IV, indicating fibrillar tissue and amorphous tissue with fluid[80], was marginal in healthy active young mature tendons. Previous research using UTC in an asymptomatic older population, with a mean age of 43,6 years, found less type II tendon bundles (with the same amount of type I tendon bundles).[80] This might indicate that the tendons of an older population are less able to adapt to load, compared to an adolescent population. Also, the tendons of an asymptomatic older population contained more type III and IV tendon bundles[80] compared to our healthy adolescent population.

Next, the results in chapter I also indicated that the insertion of the Achilles tendons contained more type II than the midportion of the tendon. Based on the more flaring shape of the tendon at insertion[10] and compositional differences between insertion and midportion of the tendon[84], the presence of echo-type II may possibly indicate a necessary morphological, histological and functional requirement.

Finally, the normal tendon tissue characterisation showed sex-related differences, with a significantly higher amount of echo-type I fibers in the male sex. This might be explained by sex-related differences in mechanical properties[46], and the effect of estrogen on functional and structural properties of the Achilles tendon.[14, 33] Again, this sex-related difference in echo-type distribution can be considered as a normal physiological finding in this young mature population.

2. Tendon structure as a risk factor for AT

Interestingly, chapter III elucidated that the investigated structural parameters were not able to predict possible future symptoms. Regarding UTC, the presence of echo-types III and IV, representing inferior tendon quality and potentially pathology[24, 72, 80], was too small in our healthy population, therefore the results of this study do not allow a conclusion concerning early detection of future pathology based on the presence of echo-types III and IV. Consequently, this thesis was not able to identify structural markers of early presymptomatic pathology in a young adult asymptomatic population based on the UTC imaging technique. It should be noted that it is possible that the evaluation of the entire midportional volume (as used in this thesis) is not suitable. To quantify subtle differences in tendon structure that may precede the development of symptoms, the assessment of structural changes (e.g. hypoechoic regions) could be more suited.

Next, tendon thickness was prospectively investigated with ultrasonography as an early detection method for future symptoms. Our results identified that Achilles tendon thickness was not suited for detecting future pathology. Comparison to previous literature is difficult since the US devices and measurement techniques vary between studies.[20, 31, 36, 63] The examination of true tendon thickness at fixed distances from the calcaneal border (as used in this thesis) was beneficial for standardization purposes, however, it is possible that measuring maximum tendon thickness would be more suited to detect future pathology. As a consequence, the results of this study do not allow a solid conclusion concerning the increased risk of AT based on the measured tendon thickness.

3. Clinical implications and further research

A general evaluation of tendon structure by UTC or examining tendon thickness will not allow clinicians to identify individuals at risk for developing Achilles tendinopathy.

Future research should focus on well-planned sequential investigations to monitor the parameters instead of one baseline screening followed by a X year follow-up period. Also, complex systems approach has been suggested to better reflect the dynamic nature of sports injuries. This approach would require investigations of interactions between different (risk) factors, how these interactions might influence, or even alter each other to form different emergent patterns of injury. This novel view of research is stated to be the future way to perform injury prevention research.[11]

Next, further research should target an older population since this thesis only studied tendon structure of a young adult population, to exclude age-related degeneration of the Achilles tendon, and can therefore not translate the findings to an older population. Furthermore, advancing age is stated to affect tendon structural properties.[47, 57] It is known that tendons in an older population are associated with increased tendon degeneration and, since this has been shown to decrease load tolerance, this degeneration is stated to play an major role in tendon pathology.[45] Furthermore, previous research using UTC in an asymptomatic older population (mean age of 43,6 y) found the same amount of type I tendon bundles, but less type II and more type III and IV tendon bundles[80], indicating that tendons of elderly persons might show differences in echopattern compared to young mature tendons. It is therefore possible that UTC evaluation in an older population would be more suited as detection method for future symptoms and therefore this should be studied prospectively. Also, the presence of structural changes (e.g. hypoechoic regions) should also be examined since the evaluation of the entire midportional volume is possibly not sensitive enough to predict future symptoms.

What is the importance of tendon blood flow in the underlying mechanism of AT and could this be established as a risk factor for AT?

1. What Is the importance of blood flow in the development of AT?

In the pathophysiological process of tendinopathy, the importance of blood flow has been previously described. As stated, overuse is generally considered to induce the condition because if the demands on the tendon are higher than can be managed, pathology occurs.[22] If (excessive) load is placed on the tendon, the body reacts with a repair process, which is adequate in most cases, but inadequate in patients developing tendinopathy. This induces a pathogenic cascade that causes a repetitive cycle of inadequate collagen and matrix production, tenocyte disruption, a further decrease in collagen and matrix and an increased vulnerability to further damage.[81] Interestingly, an inadequate blood supply is reported as a possible cause for this inadequate healing since hypovascular tendon tissue, possibly correlated with the pathogenetic role of hypoxia, is associated with tendon degeneration and subsequent development of symptomatic tendinopathy.[28, 40, 66, 81]

Although blood supply is frequently suggested as a very important intrinsic frisk factor in the development of AT [45], to our knowledge, the vascular response to activity has never been prospectively examined as risk factor for AT. This vascular response to activity, also called exercise hyperaemia, is well-established in skeletal muscle where its importance is underlined to couple blood flow with metabolism.[12, 19, 39, 74] Therefore, this thesis prospectively examined the vascular response to

activity and identified a diminished tendon vascular response to activity as a risk factor for the development of AT. This diminished tendon vascular response to activity might not be sufficient to fulfil the increased metabolic demand that is associated with physical activity. It should be noted that blood flow in rest showed no significant association with the development of AT. This could possibly indicate that the baseline tendon vascularization is adequate[2] but if the metabolic demand increases due to physical activity[27], the inability to fulfil this increased metabolic demand can increase the risk for pathology. This assumption is supported by previous studies, in skeletal muscular tissue, that stated that a diminished vascular response to activity leads to inadequate tissue oxygen delivery and metabolic dysregulation.[18, 23, 34, 39, 74] Furthermore, in tendon tissue, it is shown that inadequate tissue oxygen delivery and metabolic dysregulation can cause weakening of tendon structure and eventually AT.[1, 66, 67, 70] Also, it has been suggested that an inadequate blood supply leads to anaerobic conditions in the tendon. This could be a hazardous situation, which is supported by studies that found an association between higher lactate levels and pathological Achilles tendons.[3] However the cause-effect relationship is not clear.

2. Demographic influence on tendon blood flow

In chapter IV it was shown that a diminished increase in blood flow after physical activity, that has been identified as a risk factor for the development of AT, was influenced by demographical factors. More specifically, the increase in Achilles tendon blood flow immediately after physical activity is lower in an older population and the male participants in the older population showed a lower increase in tendon blood flow compared to their female counterparts. Interestingly, the current literature suggests that risk factors in the development of AT may include male sex [37, 43] and advancing age.[43, 44, 87] However, these statements are based on observations of higher incidence of AT in middle-aged men and not based on prospective studies.[62] This has previously been explained by the association of age with increased prevalence of tendon degeneration.[68] These degenerative changes included decreased cellularity, increased glycosaminoglycan content and lack of fiber organization.[45] Interestingly, it should be mentioned that advancing age is also associated with decreased vascularity [5, 53, 75] and with a decreased vascular response to activity in muscles.[9] Based on the results of this thesis, it might be possible that this lower vascular response to activity initiates the tendon degeneration. Indeed, research in muscular tissue has shown that a lower vascular response leads to inadequate tissue oxygen delivery and metabolic dysregulation[19, 23, 34, 38, 74], and these factors cause inadequate tissue repair.[2] Therefore, the degeneration of tendon structure could be a consequence of this inadequate repair [49, 78], and might be seen later in the pathological process. This assumption is

in line with previous research that found an association between a decrease in microcirculation and the onset of tendon degeneration[28], more specifically: a decrease in vascular tendon supply restricts the collagen matrix synthesis and leads to weakening of normal tendon structure.[66, 67]

Next, our results showed that the male participants in the older population showed a lower increase in tendon blood flow compared to their female counterparts. The previously suggested increased risk for male sex has been explained by the presence of estrogen in females, since estrogen is suggested to affect tendon structure positively.[21] Remarkably, estrogen has also been shown to positively affect the vascular system.[83] Since sex-related differences in the ageing vascular function and structure have been established, as illustrated in Fig. *1*, with premenopausal women having better overall vascular function compared to men of the same age [52], this might explain the sex-related difference found in the vascular response to activity. Based on the results of this thesis, it seems plausible that this lower blood flow response to activity could indirectly explain the higher incidence of AT in middle-aged men, with the real underlying factors possibly being age- and sex-related differences in vascular response to activity.

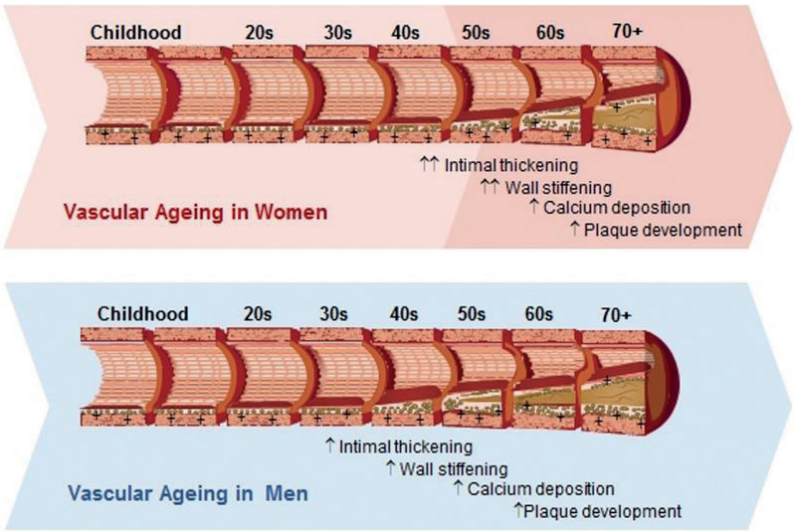


Fig. 1 Patterns of vascular ageing in women and men (by Merz et al.[52]).

It is important to mention that in chapter III, the female sex was identified at higher risk to develop AT, whereas chapter IV indicated that the male older population showed a lower vascular response to activity. The younger population in chapter IV did not show a sex-related difference in the increase in blood flow after activity, and neither did the young mature population in chapter III. Therefore, the increased risk for the female sex, defined in chapter III, is not associated with the vascular response to activity. A possible explanation for the increased risk for the female students was that significantly less extramural sports participation was registered in the female students compared to the male students, but no difference was made for sex in their educational sports program. Therefore it seems possible that the female students were less resistant to withstand this increased load. The results of this thesis indicate that caution might be needed when a similar physical activity load program is imposed on young mature males and females.

3. Clinical implications and further research

van Mechelen et al.[79] designed a model for the development of preventive interventions for injuries and clearly showed that knowledge of risk factors is essential for the development of preventive measures. Furthermore, next to their crucial role in (secondary) prevention [51], these risk factors are likely to play an important role in the response to treatment in tendinopathy.[22] Therefore, as clinical implication, it seems evident that strategies to modify this diminished vascular response to activity should be considered to include in rehabilitation and as (secondary) preventive measures.

In addition, it seems logical that middle-aged men and others with a lower increase in blood flow after physical activity need more emphasize on warm-up in order to improve the vascular response to activity and consequently reduce the risk for developing AT. Furthermore, stretching does not significantly affect Achilles tendon blood flow, and should not replace an aerobe warm-up activity to prepare the body for sports participation.

Logically, further research should focus on investigating strategies to modify this diminished vascular response to activity in the Achilles tendon. The effect of training programs on the vascular response to activity in tendons should be investigated since it is stated that physical activity has the potential to offset the age-related decline in blood flow in skeletal muscles during exercise, thereby improving oxygen delivery and an enhanced energy production from oxidative metabolism. This effect has been showed in skeletal muscles after a period of aerobic high-intensity exercise training [8, 60], but to the best of our knowledge, this has not yet been studied in tendons. Other possible strategies to increase this vascular response to

activity might be the use of prostaglandins, vibration techniques, massage, heat, beetroot juice, nitroglycerin plasters etc. because of their increasing effect on blood flow (found in skin or skeletal muscles).[13, 41, 59, 64, 69, 82, 85] However, further research is needed to investigate the vascular response of these latter techniques in the Achilles tendon since current literature is limited to changes in blood flow in the skin surface or in skeletal muscles. The implementation of these strategies to increase this vascular response to activity is especially important for middle-aged men and patients with a history of complaints, but also in athletes, because of the high loads on the tendons, to ensure the fulfillment of an increased metabolic demand during activity and an adequate repair after loading, thereby preventing pathology.

Another interesting topic for further research is the correlation between the vascular response to activity and Achilles tendon structure. Since tendon structure, more specifically tendon degeneration, has been shown to decrease load tolerance, this plays an major role in tendon pathology [45], and based on the results of this thesis, it might be possible that a diminished vascular response to activity initiates the tendon degeneration. Therefore, the cause-effect relationship of the vascular response to activity and tendon degeneration should be further studied. Also, the possible involvement of Kager's fat pad and the compression of the Plantaris tendon should be further examined. Furthermore, the potential sex-related differences in UTC tendon structure should be established in this older population.

Finally, sports activity should start within 5 min after warm-up since the results in chapter IV showed a significant decrease of tendon blood flow already 5 minutes after physical activity compared to immediately after and this was seen in all participants.

What is the importance of biomechanics in the underlying mechanism of AT and could this be established as a risk factor for AT?

1. Whipping phenomenon: what's all the fuzz about?

The whipping phenomenon is a widely cited hypothesis regarding the etiology of AT and suggests that, in excessive pronation, the transverse plain rotation of the tendon is accentuated and causes the Achilles tendon to undergo a "whipping" action which causes a decrease in blood flow.[17] The intimate relationship of the calcaneus with the distal shaft of the tibia have led researchers to speculate that the function of an overpronated foot, particularly during weight-bearing activities (e.g. the support phase of running), may alter the course of the Achilles tendon fibers, impairing its blood supply by wringing out its blood vessels.[17, 76] This ischemic

suffocation may be a predisposing factor to the Achilles tendon hypovascularization – degeneration – injury cycle.[17, 76] Although foot pronation is a factor that has been frequently proposed as an intrinsic causative factor of Achilles tendon pathology[17, 73], there was no conclusive evidence of the injury mechanism behind this whipping hypothesis. Therefore, in chapter II, evidence was provided to confirm that the more eversion excursion observed during running, the lower the increase in blood flow after running. This finding supports, at least in part, the speculations addressed by Smart et al.[76] and Clement et al.[17] with regard to the effect of foot pronation on Achilles tendon blood flow. It should be noted that the term 'pronation' is defined as a triplanar motion involving a combination of rearfoot eversion, dorsiflexion and abduction of the foot[77], but is often used in literature to refer to the measured amount of eversion.[58] The finding that more eversion excursion during running is associated to a lower increase in blood flow after running only partly supports the whipping phenomenon since this cross-sectional study showed an association between blood flow and pronation but did not provide evidence to identify foot pronation as a risk factor for the development of AT, nor was the suggested rotation inside the Achilles tendon measured.

2. Foot posture as a risk factor for AT

Altered gait kinematics and kinetics, particularly foot pronation, are frequently hypothesized to be a risk factor for AT although prospective studies are lacking.[55] Therefore, this thesis prospectively examined a pronated foot posture, defined by the Foot Posture Index, as a risk for the development of AT in chapter III. Surprisingly, this thesis was not able to identify this pronated foot posture as a risk for the development of AT. This might be explained by the measurement technique since it is possible that Foot Posture Index evaluations were not sensitive enough to predict the dynamic pronation of the participants during running[42], as referred to in the above described whipping hypothesis. Another possible explanation is that the foot pronation is not a risk factor in itself, but is associated with the vascular response to activity that is identified as risk factor for AT. More specifically, chapter II found that the more eversion excursion during running, the lower the vascular response to activity in the Achilles tendon and chapter III identified a diminished vascular response to activity as a risk factor for the development of AT. It is therefore possible that pronation is a contributing factor, increasing the risk for developing AT, particularly in individuals with a diminished vascular response to activity. It is possible that pronation does not increase the risk for AT in individuals with an adequate vascular response to activity, but in individuals with a diminished vascular response the amount of pronation could be a contributing factor, increasing the risk for AT. This might explain why foot pronation has never been prospectively identified as a risk factor in the development of AT, but is based on cross-sectional

studies associated with AT since they found that subjects with AT showed greater pronation during running than controls.[25, 50, 73]

3. Clinical implications and further research

Evaluating the Foot Posture Index will not allow clinicians to identify individuals at risk for developing AT. Furthermore, based on the relationship between pronation and the vascular response to activity established in chapter II and the identification of the vascular response to activity as a risk factor for AT in chapter III, it might be possible that anti-pronation measures, such as taping, orthoses or antipronation shoes[30, 48, 56, 86], might have a positive effect on the development of AT, particularly in individuals with a diminished vascular response to activity since it seems possible that pronation does not increase the risk for AT in individuals with an adequate vascular response to activity, but in individuals with a diminished vascular response the amount of pronation could be a contributing factor, possibly increasing the risk for AT. Then, anti-pronation measures might be useful, but further research is needed to investigate this. could be useful in managing and preventing AT. Also, plantar intrinsic foot muscles exercises[54], improving hip muscle strength[32] and running retraining[7] may positively influence the vascular response to activity since these interventions are suggested to affect a contributing factor, namely preventing excessive foot pronation.

Further research should focus on prospectively investigating the role of dynamic pronation during weight-bearing activities in the development of AT. Also, the interaction between dynamic pronation during weight-bearing activity and the vascular response to activity, how interactions might influence, or even alter each other to form different emergent patterns of injury, should be further investigated.

Strengths and limitations of this thesis

The first and most important limitation of this study was that only healthy young students were included in the prospective study. Consequently, no extrapolation of the data could be done towards an older population. However, this population was targeted to exclude age-related degeneration of the tendon. It should be noted that pubertal status was not measured to check maturity. Also, only healthy tendons were included, however, no US imaging of the tendon structure was performed to exclude/include subjects. Another limitation is that other possible risk factors for AT (e.g. psychosocial and personal factors) were not included in this study. Also, prospectively examining running kinematics could have been of added value in order to investigate the role of these running kinematics in the development of AT. The randomized order of the measurements of Achilles tendon thickness and blood flow after activity in the prospective study could also be seen as a limitation since the increase in blood flow after activity is followed by a quick recovery to baseline.[71] However, we added timing of blood flow measurements as a confounder to our statistical analysis, which we believe overcame this limitation. Moreover, in our cross-sectional study designs, blood flow measurements were performed immediately after the physical activity. In this study, we have used the oxygen-to-see to perform the blood flow measurements, which does not allow a real-time measurement. There is no golden standard for measuring tendon blood flow[15], and this non-invasive device has shown to be valid and reliable.[29] Furthermore, since an association between the decrease in Achilles tendon blood flow and tendon degeneration has been shown[28], measuring tendon structure in an older population could have given this study an added value. Also, menopause was not taken into account when women were recruited. Next, it is possible that the general UTC evaluation, whereby the midportional volume 2-6 cm proximal of the calcaneus was assessed, is not suitable to detect small structural changes in the tendon. Therefore, the assessment of possibly present structural changes, e.g. hypoechoic regions, rather than assessing the entire midportion, could be more suited as a predictor of future pathology. Also, in the first study, the large amount of independent variables included in the statistical analysis led to an increased possibility of a type I error. However, a Bonferroni correction was not applicable as the variables that were evaluated in this study were strongly correlated. Therefore, unadjusted p-values were reported, according to the recommendation of Altman et al. (2000).[4] As a final limitation, an expansion of the sample size is always beneficial. The number of events (27 subjects with AT) in chapter III allowed the detection of moderate to strong associations between the risk factor and injury risk. However, small to moderate associations between our investigated parameters and AT could not be identified since this would need about 200 injured subjects.[6]

In addition, well-planned sequential investigations to monitor the investigated parameters (e.g. weekly) could be more suited as study design to investigate these risk factors. And the implementation of the complex systems approach, e.g. investigating interactions between different (risk) factors, could be more efficient in injury prevention research.[11]

Despite several limitations, this thesis included one of the few prospective studies that investigated risk factors in the development of AT. Mainly, this is the first large-scale study that investigated the role of the vascular response to activity, foot posture, and UTC tendon structure as possible risk factors in the development of AT. Although blood flow has been frequently suggested as a risk factor in the development of AT, prospective studies were lacking and the need for prospective study designs has already been repeatedly described in literature.[16] Since only longitudinal studies can determine causative relationships[62], the prospective cohort study design can thus certainly be seen as a strength. Also, the number of participants, making this a large-scale study and the second largest prospective study investigating risk factors for AT, is of great value. Additionally, the multilevel injury registration method with the cooperation of high educated physicians in sports medicine was also a strength within the study design. Furthermore, contributing demographic, structural and biomechanical factors to the underlying mechanism of AT were examined, using adequate study designs, to gain more insight in the complex aetiology and pathophysiology of AT.

General conclusion

In early medical literature tendons are described as "virtually dead during life" based on their poor vascularization.[26] However, although vascular supply of the Achilles tendon has originally thought to be poor, more recent studies state that Achilles tendon blood flow is adequate for metabolic demands.[2] In fact, this thesis underlines the importance of tendon vascularization. The main finding of this thesis is that the vascular response to activity was identified as a risk factor in the development of AT. This is plausible since a physical activity generates an increased metabolic demand and in order to fulfil this, a necessary cardiovascular redistribution of the blood flow to the working tissue is induced.[27] Therefore, an increase in blood flow as a normal, physiological response to activity has been observed.[65] This study adds for the first time that the lower this increase in tendon blood flow, the higher the hazard for developing AT.

Furthermore, this thesis was not able to identify a pronated foot posture as a risk for the development of AT but did found an association between dynamic pronation and the vascular response to activity, partly explaining the underlying mechanism of the widely cited 'whipping phenomenon'. More specifically, the results of this thesis found that the more eversion excursion during running, the lower the vascular response to activity in the Achilles tendon.

Also, demographics were found as contributing factors to this identified vascular risk factor. The increase in Achilles tendon blood flow immediately after physical activity is lower in an older population. And the male participants in the older population showed a lower increase in tendon blood flow compared to their female counterparts, possibly explaining the higher incidence of AT in middle-aged men.

It is stated that load is a major patho-aetiological component in the development of AT, but it is modulated by intrinsic factors.[22] This underlines the importance of large scale prospective studies to identify risk factors and thereby improving the knowledge of the complex aetiology and pathophysiology of AT.

References

1. Abate M, Schiavone C, Salini V, Andia I. Occurrence of tendon pathologies in metabolic disorders. *Rheumatology (Oxford).* 2013;52(4):599-608.
2. Abate M, Silbernagel KG, Siljeholm C, et al. Pathogenesis of tendinopathies: inflammation or degeneration? *Arthritis Res Ther.* 2009;11(3):235.
3. Alfredson H, Lorentzon R. Intratendinous glutamate levels and eccentric training in chronic Achilles tendinosis: a prospective study using microdialysis technique. *Knee Surg Sports Traumatol Arthrosc.* 2003;11(3):196-199.
4. Altman DG MD, Bryant T, Gardner MJ. . Statistics with confidence: confidence intervals and statistical guidelines. *London: BMJ.* 2000.
5. Astrom M. Laser Doppler flowmetry in the assessment of tendon blood flow. *Scand J Med Sci Sports.* 2000;10(6):365-367.
6. Bahr R, Holme I. Risk factors for sports injuries--a methodological approach. *Br J Sports Med.* 2003;37(5):384-392.
7. Barton CJ, Bonanno DR, Carr J, et al. Running retraining to treat lower limb injuries: a mixed-methods study of current evidence synthesised with expert opinion. *Br J Sports Med.* 2016;50(9):513-526.
8. Beere PA, Russell SD, Morey MC, Kitzman DW, Higginbotham MB. Aerobic exercise training can reverse age-related peripheral circulatory changes in healthy older men. *Circulation.* 1999;100(10):1085-1094.
9. Behnke BJ, Prisby RD, Lesniewski LA, Donato AJ, Olin HM, Delp MD. Influence of ageing and physical activity on vascular morphology in rat skeletal muscle. *J Physiol.* 2006;575(Pt 2):617-626.
10. Benjamin M, Kaiser E, Milz S. Structure-function relationships in tendons: a review. *J Anat.* 2008; 212(3):211-228.
11. Bittencourt NF, Meeuwisse WH, Mendonca LD, Nettel-Aguirre A, Ocarino JM, Fonseca ST. Complex systems approach for sports injuries: moving from risk factor identification to injury pattern recognition-narrative review and new concept. *Br J Sports Med.* 2016.
12. Boushel R, Langberg H, Green S, Skovgaard D, Bulow J, Kjaer M. Blood flow and oxygenation in peritendinous tissue and calf muscle during dynamic exercise in humans. *J Physiol.* 2000;524 Pt 1:305-313.
13. Boushel R, Langberg H, Risum N, Kjaer M. Regulation of blood flow by prostaglandins. *Curr Vasc Pharmacol.* 2004;2(2):191-197.
14. Bryant AL, Clark RA, Bartold S, et al. Effects of estrogen on the mechanical behavior of the human Achilles tendon in vivo. *J Appl Physiol (1985).* 2008;105(4):1035-1043.
15. Casey DP, Curry TB, Joyner MJ. Measuring muscle blood flow: a key link between systemic and regional metabolism. *Curr Opin Clin Nutr Metab Care.* 2008;11(5):580-586.
16. Cassel M, Baur H, Hirschmuller A, Carlsohn A, Frohlich K, Mayer F. Prevalence of Achilles and patellar tendinopathy and their association to intratendinous changes in adolescent athletes. *Scand J Med Sci Sports.* 2015;25(3):e310-318.
17. Clement DB, Taunton JE, Smart GW. Achilles tendinitis and peritendinitis: etiology and treatment. *Am J Sports Med.* 1984;12(3):179-184.
18. Clifford PS. Skeletal muscle vasodilatation at the onset of exercise. *J Physiol.* 2007;583(Pt 3):825-833.
19. Clifford PS, Hellsten Y. Vasodilatory mechanisms in contracting skeletal muscle. *J Appl Physiol (1985).* 2004;97(1):393-403.
20. Comin J, Cook JL, Malliaras P, et al. The prevalence and clinical significance of sonographic tendon abnormalities in asymptomatic ballet dancers: a 24-month longitudinal study. *Br J Sports Med.* 2013;47(2):89-92.
21. Cook JL, Bass SL, Black JE. Hormone therapy is associated with smaller Achilles tendon diameter in active post-menopausal women. *Scand J Med Sci Sports.* 2007;17(2):128-132.
22. Cook JL, Purdam CR. Is tendon pathology a continuum? A pathology model to explain the clinical presentation of load-induced tendinopathy. *Br J Sports Med.* 2009;43(6):409-416.
23. DeLorey DS, Kowalchuk JM, Paterson DH. Effect of age on O(2) uptake kinetics and the adaptation of muscle deoxygenation at the onset of moderate-intensity cycling exercise. *J Appl Physiol (1985).* 2004;97(1):165-172.

24. Docking SI, Rosengarten SD, Daffy J, Cook J. Structural integrity is decreased in both Achilles tendons in people with unilateral Achilles tendinopathy. *J Sci Med Sport.* 2015;18(4):383-387.
25. Donoghue OA, Harrison AJ, Laxton P, Jones RK. Orthotic control of rear foot and lower limb motion during running in participants with chronic Achilles tendon injury. *Sports Biomech.* 2008;7(2):194-205.
26. Edwards DA. The blood supply and lymphatic drainage of tendons. *J Anat.* 1946;80(Pt 3):147-152 142.
27. Fadel PJ. Reflex control of the circulation during exercise. *Scand J Med Sci Sports.* 2015;25 Suppl 4:74-82.
28. Fenwick SA, Curry V, Harrall RL, Hazleman BL, Hackney R, Riley GP. Expression of transforming growth factor-beta isoforms and their receptors in chronic tendinosis. *J Anat.* 2001;199(Pt 3):231-240.
29. Forst T, Hohberg C, Tarakci E, Forst S, Kann P, Pfutzner A. Reliability of lightguide spectrophotometry (O2C) for the investigation of skin tissue microvascular blood flow and tissue oxygen supply in diabetic and nondiabetic subjects. *J Diabetes Sci Technol.* 2008;2(6):1151-1156.
30. Franettovich M, Chapman A, Blanch P, Vicenzino B. A physiological and psychological basis for anti-pronation taping from a critical review of the literature. *Sports Med.* 2008;38(8):617-631.
31. Giombini A, Dragoni S, Di Cesare A, Di Cesare M, Del Buono A, Maffulli N. Asymptomatic Achilles, patellar, and quadriceps tendinopathy: a longitudinal clinical and ultrasonographic study in elite fencers. *Scand J Med Sci Sports.* 2013;23(3):311-316.
32. Habets B, Smits HW, Backx FJG, van Cingel REH, Huisstede BMA. Hip muscle strength is decreased in middle-aged recreational male athletes with midportion Achilles tendinopathy: A cross-sectional study. *Phys Ther Sport.* 2017;25:55-61.
33. Hansen M, Kjaer M. Influence of sex and estrogen on musculotendinous protein turnover at rest and after exercise. *Exerc Sport Sci Rev.* 2014;42(4):183-192.
34. Hearon CM, Jr., Dinenno FA. Regulation of skeletal muscle blood flow during exercise in ageing humans. *J Physiol.* 2016;594(8):2261-2273.
35. Heinemeier KM, Schjerling P, Heinemeier J, Magnusson SP, Kjaer M. Lack of tissue renewal in human adult Achilles tendon is revealed by nuclear bomb (14)C. *FASEB J.* 2013;27(5):2074-2079.
36. Jhingan S, Perry M, O'Driscoll G, et al. Thicker Achilles tendons are a risk factor to develop Achilles tendinopathy in elite professional soccer players. *Muscles Ligaments Tendons J.* 2011;1(2):51-56.
37. Jones BH, Bovee MW, Harris JM, 3rd, Cowan DN. Intrinsic risk factors for exercise-related injuries among male and female army trainees. *Am J Sports Med.* 1993;21(5):705-710.
38. Joyner MJ, Casey DP. Regulation of increased blood flow (hyperemia) to muscles during exercise: a hierarchy of competing physiological needs. *Physiol Rev.* 2015;95(2):549-601.
39. Joyner MJ, Wilkins BW. Exercise hyperaemia: is anything obligatory but the hyperaemia? *J Physiol.* 2007;583(Pt 3):855-860.
40. Jozsa L, Kannus P. Histopathological findings in spontaneous tendon ruptures. *Scand J Med Sci Sports.* 1997;7(2):113-118.
41. Kubo K, Ikebukuro T. Blood circulation of patellar and achilles tendons during contractions and heating. *Med Sci Sports Exerc.* 2012;44(11):2111-2117.
42. Langley B, Cramp M, Morrison SC. Selected static foot assessments do not predict medial longitudinal arch motion during running. *J Foot Ankle Res.* 2015;8:56.
43. Longo UG, Ronga M, Maffulli N. Achilles tendinopathy. *Sports Med Arthrosc Rev.* 2009;17(2):112-126.
44. Maffulli N, Wong J, Almekinders LC. Types and epidemiology of tendinopathy. *Clin Sports Med.* 2003;22(4):675-692.
45. Magnan B, Bondi M, Pierantoni S, Samaila E. The pathogenesis of Achilles tendinopathy: a systematic review. *Foot Ankle Surg.* 2014;20(3):154-159.
46. Magnusson SP, Hansen M, Langberg H, et al. The adaptability of tendon to loading differs in men and women. *Int J Exp Pathol.* 2007;88(4):237-240.
47. Magnusson SP, Hansen P, Kjaer M. Tendon properties in relation to muscular activity and physical training. *Scand J Med Sci Sports.* 2003;13(4):211-223.
48. Malisoux L, Chambon N, Delattre N, Gueguen N, Urhausen A, Theisen D. Injury risk in runners using standard or motion control shoes: a randomised controlled trial with participant and assessor blinding. *Br J Sports Med.* 2016;50(8):481-487.

49. Marquez-Arabia WH, Gomez-Hoyos J, Gomez M, et al. Influence of Aging on Microvascular Supply of the Gluteus Medius Tendon: A Cadaveric and Histologic Study. *Arthroscopy.* 2017;33(7):1354-1360.
50. McCrory JL, Martin DF, Lowery RB, et al. Etiologic factors associated with Achilles tendinitis in runners. *Med Sci Sports Exerc.* 1999;31(10):1374-1381.
51. Meeuwisse WH. Predictability of sports injuries. What is the epidemiological evidence? *Sports Med.* 1991;12(1):8-15.
52. Merz AA, Cheng S. Sex differences in cardiovascular ageing. *Heart.* 2016;102(11):825-831.
53. Moller A, Astron M, Westlin N. Increasing incidence of Achilles tendon rupture. *Acta Orthop Scand.* 1996;67(5):479-481.
54. Mulligan EP, Cook PG. Effect of plantar intrinsic muscle training on medial longitudinal arch morphology and dynamic function. *Man Ther.* 2013;18(5):425-430.
55. Munteanu SE, Barton CJ. Lower limb biomechanics during running in individuals with achilles tendinopathy: a systematic review. *J Foot Ankle Res.* 2011;4:15.
56. Munteanu SE, Scott LA, Bonanno DR, et al. Effectiveness of customised foot orthoses for Achilles tendinopathy: a randomised controlled trial. *Br J Sports Med.* 2015;49(15):989-994.
57. Narici MV, Maffulli N, Maganaris CN. Ageing of human muscles and tendons. *Disabil Rehabil.* 2008;30(20-22):1548-1554.
58. Nigg BM. *Biomechanics of sports shoes*. Canada: Topline Printing Inc; 2010.
59. Notay K, Incognito AV, Millar PJ. Acute beetroot juice supplementation on sympathetic nerve activity: a randomized, double-blind, placebo-controlled proof-of-concept study. *Am J Physiol Heart Circ Physiol.* 2017;313(1):H59-H65.
60. Nyberg M, Gliemann L, Hellsten Y. Vascular function in health, hypertension, and diabetes: effect of physical activity on skeletal muscle microcirculation. *Scand J Med Sci Sports.* 2015;25 Suppl 4:60-73.
61. O'Brien TD, Reeves ND, Baltzopoulos V, Jones DA, Maganaris CN. Mechanical properties of the patellar tendon in adults and children. *J Biomech.* 2010;43(6):1190-1195.
62. O'Neill S, Watson PJ, Barry S. A Delphi Study of Risk Factors for Achilles Tendinopathy- Opinions of World Tendon Experts. *Int J Sports Phys Ther.* 2016;11(5):684-697.
63. Ooi CC, Schneider ME, Malliaras P, et al. Sonoelastography of the Achilles Tendon: Prevalence and Prognostic Value Among Asymptomatic Elite Australian Rules Football Players. *Clin J Sport Med.* 2016;26(4):299-306.
64. Osterspey A, Jansen W, Ulbrich T, Simon P, Tauchert M, Hilger HH. [Effect of nitroglycerin plasters on hemodynamics and stress tolerance of patients with coronary heart disease]. *Dtsch Med Wochenschr.* 1984;109(18):714-717.
65. Pingel J, Harrison A, Simonsen L, Suetta C, Bulow J, Langberg H. The microvascular volume of the Achilles tendon is increased in patients with tendinopathy at rest and after a 1-hour treadmill run. *Am J Sports Med.* 2013;41(10):2400-2408.
66. Pufe T, Petersen WJ, Mentlein R, Tillmann BN. The role of vasculature and angiogenesis for the pathogenesis of degenerative tendons disease. *Scand J Med Sci Sports.* 2005;15(4):211-222.
67. Rahim M, El Khoury LY, Raleigh SM, et al. Human Genetic Variation, Sport and Exercise Medicine, and Achilles Tendinopathy: Role for Angiogenesis-Associated Genes. *OMICS.* 2016;20(9):520-527.
68. Rees JD, Wilson AM, Wolman RL. Current concepts in the management of tendon disorders. *Rheumatology (Oxford).* 2006;45(5):508-521.
69. Richards JC, Racine ML, Hearon CM, Jr., et al. Acute ingestion of dietary nitrate increases muscle blood flow via local vasodilation during handgrip exercise in young adults. *Physiol Rep.* 2018;6(2).
70. Riley G. The pathogenesis of tendinopathy. A molecular perspective. *Rheumatology (Oxford).* 2004;43(2):131-142.
71. Risch L, Cassel M, Mayer F. Acute effect of running exercise on physiological Achilles tendon blood flow. *Scand J Med Sci Sports.* 2017.
72. Rosengarten SD, Cook JL, Bryant AL, Cordy JT, Daffy J, Docking SI. Australian football players' Achilles tendons respond to game loads within 2 days: an ultrasound tissue characterisation (UTC) study. *Br J Sports Med.* 2015;49(3):183-187.

73. Ryan M, Grau S, Krauss I, Maiwald C, Taunton J, Horstmann T. Kinematic analysis of runners with achilles mid-portion tendinopathy. *Foot Ankle Int.* 2009;30(12):1190-1195.
74. Sarelius I, Pohl U. Control of muscle blood flow during exercise: local factors and integrative mechanisms. *Acta Physiol (Oxf).* 2010;199(4):349-365.
75. Sharma P, Maffulli N. Tendon injury and tendinopathy: healing and repair. *J Bone Joint Surg Am.* 2005;87(1):187-202.
76. Smart GW, Taunton JE, Clement DB. Achilles tendon disorders in runners--a review. *Med Sci Sports Exerc.* 1980;12(4):231-243.
77. Stacoff A, Reinschmidt C, Nigg BM, et al. Effects of foot orthoses on skeletal motion during running. *Clin Biomech (Bristol, Avon).* 2000;15(1):54-64.
78. Theobald P, Benjamin M, Nokes L, Pugh N. Review of the vascularisation of the human Achilles tendon. *Injury.* 2005;36(11):1267-1272.
79. van Mechelen W, Hlobil H, Kemper HC. Incidence, severity, aetiology and prevention of sports injuries. A review of concepts. *Sports Med.* 1992;14(2):82-99.
80. van Schie HT, de Vos RJ, de Jonge S, et al. Ultrasonographic tissue characterisation of human Achilles tendons: quantification of tendon structure through a novel non-invasive approach. *Br J Sports Med.* 2010;44(16):1153-1159.
81. van Sterkenburg MN, van Dijk CN. Mid-portion Achilles tendinopathy: why painful? An evidence-based philosophy. *Knee Surg Sports Traumatol Arthrosc.* 2011;19(8):1367-1375.
82. Venslauskas M, Ostasevicius V, Vilkinis P. Influence of low-frequency vibrations on blood flow improvement in human's limbs. *Biomed Mater Eng.* 2017;28(2):117-130.
83. Volterrani M, Rosano G, Coats A, Beale C, Collins P. Estrogen acutely increases peripheral blood flow in postmenopausal women. *Am J Med.* 1995;99(2):119-122.
84. Waggett AD, Ralphs JR, Kwan AP, Woodnutt D, Benjamin M. Characterization of collagens and proteoglycans at the insertion of the human Achilles tendon. *Matrix Biol.* 1998;16(8):457-470.
85. Weerapong P, Hume PA, Kolt GS. The mechanisms of massage and effects on performance, muscle recovery and injury prevention. *Sports Med.* 2005;35(3):235-256.
86. Williams S, Whatman C, Hume PA, Sheerin K. Kinesio taping in treatment and prevention of sports injuries: a meta-analysis of the evidence for its effectiveness. *Sports Med.* 2012;42(2):153-164.
87. Wise BL, Peloquin C, Choi H, Lane NE, Zhang Y. Impact of age, sex, obesity, and steroid use on quinolone-associated tendon disorders. *Am J Med.* 2012;125(12):1228 e1223-1228 e1228.

Nederlandstalige samenvatting

Een van de meest voorkomende overbelastingsletsels ter hoogte van het onderste lidmaat is een tendinopathie van de achillespees. Dit letsel komt frequent voor bij beoefenaars van atletiek en bij atleten die deelnemen aan sporten met snelle richtingsveranderingen. De hoogste incidentie van een achillespeestendinopathie wordt gezien bij mannelijke atleten van middelbare leeftijd. Omdat achillespeesletsels vaak voorkomen en zeer frequent recidiveren, hebben ze een nefaste invloed op het prestatievermogen en psychosociaal welbevinden van de atleet. Om die reden was het hoofddoel van dit doctoraatsproject het intrinsiek risicoprofiel van deze blessure diepgaander te identificeren en te definiëren.

Recent is er een nieuwe beeldvormingstechniek ontwikkeld, genaamd Ultrasound Tissue Characterization (UTC). Deze techniek heeft al aangetoond dat hij geschikt is om subtiele veranderingen in peesstructuur te kunnen waarnemen. Hij maakt namelijk gebruik van conventionele echografie om een 3D-beeld van de achillespees te creëren. Aan de hand van dit 3D-beeld wordt dan de structurele integriteit van het echopatroon bestudeerd. Zo kan er een onderscheid gemaakt worden tussen 4 verschillende echotyes. Echotype I staat voor intacte en mooi gealigneerde peesvezelbundels; echotype II wijst op minder mooi gealigneerde en meer wevende peesvezelbundels; echotype III toont voornamelijk onvolwaardig fibrillair weefsel en echotype IV is amorf weefsel met vocht. Alvorens er echter met deze nieuwe techniek een afwijkende peesstructuur kon worden vastgesteld, was er eerst nood aan normatieve data over de peesstructuur. Dit werd in de studie van het **eerste hoofdstuk** van dit doctoraatsproject onderzocht op een jonge, gezonde en sportieve populatie. De pezen van jonge, gezonde proefpersonen hadden een hoog percentage type II vezels, wat aantoont dat de aanwezigheid van deze type II vezels een normaal fysiologisch gegeven is bij gezonde, sportieve jongeren en dus niet op pathologie wijst. Bovendien geven de resultaten van deze studie aan dat er structurele verschillen zijn in peesstructuur op basis van geslacht. Er zijn meer type II vezels bij de vrouwelijke proefpersonen dan bij de mannen.

Het **tweede hoofdstuk** van dit doctoraatsproject bestudeerde uitvoerig volgende frequent gesuggereerde oorzaak voor het oplopen van een achillespeestendinopathie: een afwijkend looppatroon zorgt voor een verhoogde torsie van de achillespees, waardoor de doorbloeding van de pees gecomprimeerd wordt en er letsels ontstaan. Voorafgaande studies hebben zich gefocust op de relatie tussen dit afwijkend looppatroon (meer bepaald overmatige pronatie tijdens het lopen) en achillespeesletsels. Omdat het onderliggende mechanisme hiervan nog nooit was onderzocht, werd juist die relatie tussen de mate van pronatie tijdens het lopen en de door-

bloeding van de achillespees het gespreksonderwerp van ons tweede hoofdstuk. Hiervoor werden ervaren lopers getest en aan de hand van een kinematicastudie werd hun looppatroon in het frontale en sagittale vlak onderzocht. De doorbloeding van hun achillespees werd voor en na deze loopactiviteit gemeten met het oxygen-to-see toestel. De resultaten van deze studie toonden aan dat de mate van pronatie tijdens het lopen een significant effect had op de doorbloeding van de achillespees: hoe meer pronatie werd waargenomen, hoe minder de doorbloeding tijdens de inspanning steeg. Hierdoor is het gebruik van anti-pronatie maatregelen, zoals steunzolen en tape, wellicht zinvol om achillespeesletsels te voorkomen en te behandelen.

In **het derde hoofdstuk** werden mogelijke risicofactoren voor het oplopen van een achillespeestendinopathie prospectief onderzocht. Deze grootschalige studie toonde aan dat het minder stijgen van de achillespeesdoorbloeding na fysieke activiteit als risicofactor kon geïdentificeerd worden voor het oplopen van een achillespeesletsel. Met andere woorden als de doorbloeding van de achillespees minder stijgt na het sporten is er meer kans om een achillespeesletsel op te lopen. Het is goed mogelijk dat een adequate stijging in doorbloeding noodzakelijk is om aan de gestegen metabolische eisen van de sport te kunnen voldoen. Bovendien bleek dat vrouwen meer kans hebben om een achillespeesletsel op te lopen als ze een programma met gelijkaardige belasting als bij mannen ondergaan. De stand van de voet kon echter niet geïdentificeerd worden als risicofactor. Een generale UTC-evaluatie of het opmeten van de dikte van de achillespees is eveneens geen geschikte methode om aan snelle detectie van een achillespeestendinopathie te kunnen doen.

De demografische invloeden op de geïdentificeerde risicofactor voor het oplopen van een achillespeestendinopathie, namelijk de inadequate stijging van de achillespeesdoorbloeding na fysieke activiteit, werden op hun beurt onderzocht in **het vierde hoofdstuk**. Hier bleek dat de oudere populatie een minder goede vasculaire respons heeft op fysieke activiteit. Bovendien is er een verschil in geslacht in de oudere populatie: de vasculaire respons was lager bij de mannen dan bij de vrouwen. Dit zou eventueel kunnen verklaren waarom de hoogste incidentie van achillespeestendinopathieën gezien wordt bij mannelijke atleten van middelbare leeftijd.

We besluiten met de vaststelling dat het zinvol is om op basis van deze geïdentificeerde risicofactor en de gevonden associaties preventieve maatregelen te nemen. Immers, het opstellen van een accuraat en individueel (secundair) preventief programma is het ultieme doel dat zowel door onderzoekers als (para)medici werkzaam in blessurepreventie, moet nagestreefd worden.

List of abbreviations

AT	Achilles Tendinopathy
AU	Arbitrary units
BMI	Body Mass Index
CI	Confidence Interval
Etc.	et cetera
FPI	Foot Posture Index
Hz	Hertz
ICC	Intraclass Correlation Coefficient
i.e.	id est
LE	Lower Extremity
MD	Doctor of Medicine
MDC	Minimal detectable change
O2C	Oxygen-to-see
PhD	Philosophical Doctor
ROI	Region Of Interest
ROM	Range Of Motion
RPE	Rate of Perceived Exertion
Rpm	Revolutions per minute
SD	Standard Deviation
SEM	Standard Error of the Measurement
SPSS	Statistical Package for the Social Sciences
US	Ultrasound
UTC	Ultrasound Tissue Characterization
UZ	Universitair Ziekenhuis
2D	two-dimensional
EUS	Ultrasound elastography

Acknowledgements | Dankwoord

Dit doctoraat was een zeer waardevol leerproces gedurende de afgelopen 6 jaar. Nu de laatste hand aan mijn doctoraatsthesis gelegd is, neem ik graag even de tijd om stil te staan bij de mensen die de afgelopen jaren bewust of onbewust hebben bijgedragen tot de realisatie hiervan.

Eerst en vooral wil ik mijn promotoren oprecht bedanken. Het was geen doorsnee traject, waardoor ik het genoegen had om met verschillende promotoren te mogen werken. Dit project is opgestart onder leiding van Nele Mahieu. Beste Nele, een oprechte dankjewel voor het vertrouwen dat je mij gegeven hebt. Ik kon altijd bij jou terecht en je hielp me steeds met veel enthousiasme. Jouw steun en begeleiding hebben zeer veel voor mij betekend.

Na 2 jaar werd Prof. dr. Erik Witvrouw mijn promotor, maar aangezien hij nog in Qatar verbleef op dat moment, werd ik tijdelijk toevertrouwd aan Prof. dr. Tine Willems. Beste Tine, ik heb een enorme bewondering voor jouw efficiënte en directe aanpak. Jouw begeleiding bij mijn analyses was van onschatbare waarde, bedankt om jouw kennis met mij te delen, ik heb heel veel van jou geleerd.

Beste Erik, de afgelopen jaren heb ik de eer gehad om intens met jou te mogen samenwerken om dit project tot een goed einde te brengen. Je stond steeds voor mij klaar met constructieve feedback en goede ideeën. Jouw unieke manier van begeleiden stimuleerde me om alle uitdagingen aan te gaan. Bedankt voor de waardevolle discussies, het gekregen vertrouwen en de fijne samenwerking.

Ook had ik graag een oprechte dankjewel gezegd aan mijn copromotor Prof. dr. Dirk De Clercq. Beste Dirk, ondanks je drukke agenda nam je steeds de tijd om mij van constructieve en waardevolle feedback te voorzien. Ook veel dank om steeds te supporteren als ik een presentatie had of een artikel had ingestuurd. Dat heb ik enorm geapprecieerd.

Beste promotoren, dankzij jullie indrukwekkende kennis, gedrevenheid en complementaire begeleiding is dit doctoraat het afgewerkte geheel dat het nu is.

Veel dank gaat uit naar de leden van mijn begeleidingscommissie: Prof. dr. De Muynck en Prof. dr. Vanden Bossche. Bedankt voor jullie aanvullende kennis en begeleiding tijdens mijn doctoraatstraject.

Dear members of the examination board, I would like to express my sincere gratitude for your effort and time to read and evaluate my dissertation. Thank you for your interesting questions and good suggestions for improvement. I gratefully acknowledge Prof. dr. Jill Cook, Prof. dr. Ann Cools, Prof. dr. Philip Roosen, Prof. dr. Adelheid Steyaert and Prof. dr. Hans Zwerver. Ook wil ik Prof. Dr. Dirk Cambier bedanken om als voorzitter de doctoraatsverdediging te leiden.

Verder wens ik bijzonder graag al mijn thesisstudenten te bedanken die hebben meegeholpen met de testen. Zonder jullie inzet waren de studies van dit doctoraat niet mogelijk geweest. Ook een bedanking aan alle personen die hebben deelgenomen aan de onderzoeken.

Ook mijn bureaugenoten Dorien, Joke en Ruth mag ik niet vergeten. Jullie waren een enorme steun. Bedankt voor de vriendschap en de fijne momenten samen. Een warme dankjewel gaat uit naar alle REVAKI collega's voor de fijne sfeer op de werkvloer. Ik ben dankbaar dat ik deel mocht uitmaken van de 'REVAKI-familie' de afgelopen jaren. Een bijzondere dank aan het ATP personeel voor alle professionele, administratieve, technische en persoonlijke ondersteuning.

Een grote dankjewel gaat uit naar mijn vriendinnen Anouk, Eva, Joke, Lieselot en Mileen voor hun steun, meeleven, interesse en leuke afleiding van mijn werk. Ook gaat ontzettend veel dank uit naar mijn familie en schoonfamilie om steeds mee te supporteren. Heel erg bedankt de vele gezellige momenten samen en om er steeds voor mij te zijn.

Voor hun onvoorwaardelijke steun en liefde wens ik heel graag mijn ouders en mijn broer te bedanken. Mama en papa, bedankt om altijd in mij te geloven, mij door en door te kennen en mij altijd keihard aan te moedigen bij alles wat op mijn pad komt. Jullie zijn mijn grote voorbeeld. Bedankt voor alles. Lootjes, bedankt voor de vele mooie momenten samen, jullie liefde en vriendschap geven me altijd veel energie.

En tot slot wil ik graag Benjamin bedanken. Jouw intelligentie, inzet en ambitie zijn een heel grote drijfveer geweest voor mij gedurende dit traject. Bedankt voor jouw liefde, onvoorwaardelijke steun en grote vertrouwen in ons pad dat we samen bewandelen. Ik kijk enorm uit naar de rest van ons leven samen, je maakt me gelukkig.

Curriculum Vitae

General Information

Name	Evi Wezenbeek
Date of Birth	20.02.1987
Place of Birth	Ghent, Belgium
Nationality	Belgian
Private Address	Arthur van Laethemstraat 8, 9050 Gentbrugge
Phone	+32 495 54 97 66
E-mail	evi_wezenbeek@hotmail.com
Driver's license B	

Education

2013-2018	PhD in Health Sciences, Department of Rehabilitation Sciences and Physiotherapy, Faculty of Medicine and Health Sciences, Ghent university
2009	Master of Health Science in Rehabilitation Sciences and Physiotherapy, degree attained Magna cum laude

Additional courses and training

Academic Specialist Courses

Linear mixed models in SPSS (2016)
Mastering lower limb tendinopathy
Analysing running technique & running re-education
Sports injury prevention and performance training: the Swedish experience

Academic Transferrable Skills Courses

Advanced statistics (statistische analyse met behulp van SPSS – gevorderden) (2016)

Advanced Academic English: Writing Skills
Advanced Academic English: Conference Skills - Presentation Skills in English
How to Get Published
Project management
Leadership Foundation Course

Professional experience

Clinical experience

2009 - 2012	Injury Prevention Trainer talent pool JBOC Basketball
2009 - 2016	First Division Basketball player
2009 - present	Sports physiotherapist at Kiné- & SportPraktijk Wezenbeek, Melle, Belgium Sports rehabilitation Certified Spartanova Expert: Injury Prevention / Rehab / Performance

Teaching

2009 - present	Teacher at the Department of Rehabilitation Sciences and Physiotherapy, Faculty of Medicine and Health Sciences, Ghent university (1) Physiotherapy of the Lower Limb, 1st and 3rd bachelor (co-author of book) (2) Taping, 1st master (3) Sports Physiotherapy, 2nd master Teacher at the Department of Physical and Rehabilitation Medicine, Sports Medicine, Ghent University Tendinopathy and Injury prevention (author of book)
2010 - present	Teacher at Sport Vlaanderen (VTS)- Basketball Safety in sports - Preventive | Safety in sports – Curative

Publications

Wezenbeek E, Mahieu N, Willems TM, Van Tiggelen D, De Muynck M, De Clercq D, Witvrouw E. What does normal tendon structure look like? New insights into tissue characterisation in the Achilles tendon. Scand J Med Sci Sports. 2017 Jul;27(7):746-753.

Wezenbeek E, Willems TM, Mahieu N, Van Caekenberghe I, Witvrouw E, De Clercq D. Is Achilles tendon blood flow related to foot pronation? Scand J Med Sci Sports. 2017 Dec;27(12):1970-1977.

Wezenbeek E, Willems T, Mahieu N, De Muynck M, Vanden Bossche L, Steyaert A, De Clercq D, Witvrouw E. The role of the vascular and structural response to activity in the development of Achilles tendinopathy. Am J Sports Med. 2018 (In press)

International presentations

Poster presentations

International Scientific Tendinopathy Symposium - Oxford - 05/07.09.2014
IOC world conference: prevention of injury & illness in sport - Monaco - 16/18.03.2017

Oral presentations

European College of Sport Science - Amsterdam - 03.07.2014
Invited speaker at EFSMA congress (European Federation of Sports Medicine Associations) - Antwerp - 09.11.2015
Congress for Sports Medicine - Limburg - 05.12.2015
International Scientific Tendinopathy Symposium - Cape Town - 24.10.2016
Scandinavian Congress of Medicine and Science in Sports - Copenhagen - 02.02.2018
Finalist oral presentation competition

Membership and associations

2015 - present	Substitutional member of the department committee at the Department of Rehabilitation Sciences and Physiotherapy, Faculty of Medicine and Health Sciences, Ghent university_
2016 – present	Member of the education committee at the Department of Rehabilitation Sciences and Physiotherapy, Faculty of Medicine and Health Sciences, Ghent university_

Knowledge of languages

Dutch: native
English: very good
French: basic

General interests

Sports
Friends and family
Food and drinks
Travel
Animals
Reading and writing